Animals Scientific Procedures Act 1986

Non-technical summaries for project licences granted during 2020

Volume 2
July to December

Home Office

NON-TECHNICAL SUMMARY

1.Advancing the knowledge and treatment of paediatric brain cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Brain tumour, Paediatric, Cancer, Treatment

Animal types	Life stages
Mice	juvenile, adult, neonate, pregnant, embryo, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects' objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The primary objective of this project licence is to understand the origins and biology of paediatric brain tumours and to identify new treatments that can be translated into clinical practice.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Paediatric brain tumours represent the second most common cancer type to affect children but are also the most lethal. Of the 130 different kinds of paediatric brain tumour Ependymoma (EPY), Medulloblastoma (MB) and Choroid Plexus Carcinoma (CPC) are the most common and deadly solid cancers. Part of the reason for this is that treatment has not changed for more than 40 years and remains an aggressive regime of gross or partial resection surgery, radiotherapy and often chemotherapy and still results in a 30-50% death rate. Of those that do survive, the impact of such a harsh treatment program means that in the majority of cases, these children will be significantly impacted for the rest of their lives, and only 1% will achieve a college level education. Ependymoma and Choroid plexus carcinoma are also resistant to current chemotherapies and inevitably recurrence of these tumours occurs.

It is widely understood that childhood brain tumours comprise many distinct diseases each with different origins, prognoses and challenges. It fits, therefore, that each tumour type would require a different method of treatment. In order to determine the most effective treatment there is a need to first understand the biology of the disease and to develop models that faithfully recapitulate their human counterparts.

An accurate model of sonic hedgehog subtype of medulloblastoma (SHH-MB) was developed in 2004 and used to identify new treatments that could be efficiently translated to children with this tumour. However, models of other brain tumour types, such as Ependymoma, WNT-medulloblastoma and CPC, did not exist until recently. Using cross species genomics, we have now generated mouse models of each of these cancers that do not require invasive methodologies, but form tumours *in situ*. We propose in this licence to utilise these models in furthering knowledge of the mechanisms involved in initiation and progression of each tumour type, and more importantly to enrol these models into our pre-clinical mouse hospital in order to identify new treatment options that can be translated into the clinic, and ultimately reduce mortality rates and the long term side effects to the child. We will continue to use our robust and reliable, orthotopic implant (in which cells are transplanted into the brain) and patient derived xenograft (PDX, in which cells taken from human tumours are implanted into the mouse brain) models, for each tumour type, in our pre-clinical mouse hospital to explore the impact of novel therapeutic methods alongside the standard-of-care treatment. Data produced from the genetically

engineered mouse models (GEMM), orthotopic and PDX models in this setting, will provide extremely strong evidence to support the progress of these treatment methods, into human clinical trials.

What outputs do you think you will see at the end of this project?

The research outlined in this proposal aims to make much needed progress in the understanding of the origins, biology and treatment of three of the most important forms of paediatric brain tumour.

Upon completion of this project, we expect to have generated and fully characterised new genetically engineered mouse models of two of these tumour types.

Further to this we will have generated models for exploring the roles of specific genes and gene mutations in normal and tumour development (tumorigenesis). Importantly these models will have been crucial in advancing knowledge of disease relevant biology.

New treatments of childhood brain tumours are needed. Alongside the aims within this project (to develop new and repurposed drugs that may be delivered through novel localised methods) the approach laid out in this project has a significant chance of advancing effective new therapies.

One of our most important outputs will be new treatments for each of these brain tumour types that are ready for clinical trials. These will be based upon their individual biology and are therefore, specific to and effective against their respective human diseases.

Publications originating from this project and all mouse models generated will be made available to the scientific community and could thus be important for scientific advancement of other research groups.

Who or what will benefit from these outputs, and how?

In the short term the outputs of this project will provide new information that will benefit the scientific and translational research community, through peer-reviewed publication of our work. This information will not only fill gaps in current knowledge and provide a basis for further investigations into the biology of these diseases but will also provide guidance for research into other types of children's brain tumour.

As some of the tumour types in these studies are rare and therefore, with access to samples limited, we have formed international collaborative networks, who will also benefit from sharing resources. The mouse models generated will provide powerful tools for further investigations and for testing of novel treatments. Our patient derived brain tumour atlas will be an informative and detailed resource that will benefit academic and medical communities, aiding in quicker and more accurate diagnosis of patient samples.

In the mid-term, our research strategy will enable direct translation of novel treatment options from mice to human clinical trials. This will provide significant evidence regarding the efficacy of the treatment in mice and as a result, increased confidence in its success in human subjects. Benefits at this stage will be seen by the translational research scientists, clinical scientists and a small number of patients.

Our long-term aim for this project is to see clinical practice in the treatment of brain tumours change, from the current aggressive and damaging standard of care, to an effective tumour specific treatment plan that not only improves prognosis but does so with fewer, or no, lasting side effects. Thereby

improving the quality of life for patients, and their families. Following curative treatment this will further benefit healthcare practitioners, by reducing the need for life-long care and alleviating the costs associated with long-term care of survivors.

How will you look to maximise the outputs of this work?

Maximising the output of this work will be achieved through open communication and sharing of our progress throughout this project with academic, clinical and lay audiences, using a variety of forums, such as teleconferences,scientific meetings and collaborative websites.

All scientific results will be published in peer-reviewed open-access journals in a timely manner, to ensure that the information presented is widely available. At the time of publication, we will also make available the wealth of complex sequencing data and functional maps, that will be generated from this research through supplementary files and by uploading to publicly available repositories.

Following characterisation and peer-reviewed publication of the mouse models generated in this project, mice will be made available, upon request, to researchers for non-commercial research.

The researchers associated with this project will be encouraged to present their findings at scientific conferences. This will enable wide dissemination of work and promotion of further collaborations.

As our research aims to generate more effective treatment options that can be translated from mouse to human clinical trials, we believe that establishing open communication with the public, and in particular patients and their families is critical. As a result, we have actively engaged in demonstrations and lay seminars and our website will include specific elements specifically designed for public and patient engagement.

Species and numbers of animals expected to be used

- Mice: 20377

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The overall aim of this project is to gain a better understanding of the origins and biology of 3 of the most important types of paediatric brain tumour and to develop new, effective treatments that can be translated to the clinic. Using the best models and methodology is critical in achieving this aim and for this reason we will use mice for our research.

Mice are commonly used as an animal model for brain tumour research due to the following factors:

- They have similar genetics to humans

- Their embryonic and post-natal development programmes are similar to that of humans
- They have a similar central nervous system (CNS) anatomy to humans
- Mice have the most comparable neurobiology and immune system to humans
- Mouse response to treatments are the most similar of all laboratory models.

Previous research shows that the mouse models used in brain tumour research to date have demonstrated many similarities to human brain tumours and are able to mimic very closely the progression of human disease. Non-mammalian models do not share such similarities and are unable to reproduce many of the features of human brain tumours.

In vitro (cell culture) systems cannot currently replicate the complexities of the mammalian organ systems, particularly the human brain and central nervous system. Further to this, regulatory and research bodies require the preclinical assessment of potential new therapies in animal models prior to their translation to the clinic.

The scientific community has a range of techniques that enable manipulation of the mouse genome, allowing us to generate models with which we can answer specific key questions regarding brain tumour biology. The models described throughout this project represent the optimal models for the study of human childhood brain tumours and for testing the efficacy of novel therapeutics.

The studies outlined within this project that are aimed at testing novel therapeutics will involve the use of adult mice. However, protocols aimed at exploring the mechanisms of disease will include animals of varying life stages. Each stage has been specifically selected because they are the most appropriate age for the study.

Typically, what will be done to an animal used in your project?

Many of the animals used within this project will develop a brain tumour. This may be induced by one of four methods:

1. Breeding of genetically altered animals by natural breeding behaviour. This may result in the birth of animals that will spontaneously develop a brain tumour as they age, due to an activated cancer-causing gene (oncogene) or gene deletion. Alternatively, the offspring of these animals may require use of a drug, either during pregnancy or soon after birth to have the same effect. Administration after birth will require an injection on two consecutive days, after which the animal will be left to age and will be monitored for tumour development. Some pregnant females may receive up to 6 injections during pregnancy, with agents that mark developing neural cells in order to study the origins of brain tumours.

2. Some animals may receive an injection into the brain whilst they are still a developing embryo. In these cases, a pregnant female will undergo a short surgical procedure to expose the uterus and the embryos injected through the wall of the uterus. Following the injection, the uterus will be returned to the abdomen of the mother and the surgical procedure completed by sewing up the abdominal wall. The female will then be cared for and allowed to deliver the babies naturally. The offspring, once born, will be aged until they develop a tumour.

3. A small number of animals may receive an injection directly into the brain within 2 days of birth, in order to cause a tumour to develop. This will be carried out under anaesthesia.

4. Some animals on this project will develop tumours following a surgical procedure to implant tumour cells directly into the brain. This will be done on a single occasion and under general anaesthesia. Animals in these studies will only be adult animals.

The majority of these animals will be monitored for tumour development using a non-invasive imaging method, which requires light anaesthesia and injection of a substance to enable visualisation of the tumour as it grows. This will be performed on a weekly/twice weekly basis until the animals either demonstrate symptoms associated with a brain tumour or until the tumour reaches a pre-determined size before symptoms occur. They will then receive treatment for the disease. The exact timing of this will vary between the different tumour types but is performed to ensure that the suffering of any animal is kept to a minimum.

Animals that are enrolled on a treatment study will then undergo one, or a combination, of treatments that mimic the experience of a human patient in the clinic. Some of these animals will receive, as part of their treatment, newly developed but thoroughly tested, drugs and some may be delivered in new ways. These treatment options include a surgical procedure to remove the brain tumour, carried out with extreme care and under anaesthesia. Resection surgery will take no more than 45 minutes per mouse. These animals will be allowed 3-4 days to recover from surgery before being given treatment using new or current chemotherapeutic agents and/or radiotherapy.

Chemotherapy will be delivered by a variety of methods including injections, orally, or in drinking water. They may also be delivered using newly developed methods such as a biodegradable product called hydrogel. The hydrogel can be mixed with any drug and injected into the space left by the removed (resected) tumour. This will be done immediately after the tumour removal, reducing the number of procedures the animal undergoes. Alternatively, a pump may be inserted under the skin of the animal that will deliver the drug directly to the brain through a cannula, which will be installed following the removal of the tumour. Treatment studies will last no more than 3 months and animals will be monitored throughout for signs of sickness.

What are the expected impacts and/or adverse effects for the animals during your project?

Many of the animals used in this project are not expected to experience suffering. On rare occasions mice undergoing some of these procedures will exhibit post-procedural symptoms indicating pain or infection. Topical or systemic treatments for these will be immediately provided and mice will be closely and regularly monitored.

Animals that are expected to develop a brain tumour are not expected to experience suffering from the procedures used to initiate tumour formation. On rare occasions an animal may experience pain, tiredness or a head tilt following the surgery to implant tumour cells.

All animals that are expected to develop a brain tumour will be monitored closely using non-invasive imaging, in order to determine if a tumour is growing. The majority of these animals will be enrolled onto treatment studies before symptoms associated with an established brain tumour develop. However, some animals will experience symptoms including:

- Gait problems including laboured movement, wobbly movement or limb dragging.
- Excessive tiredness.
- Seizures.
- Symptoms associated with hydrocephalus (domed head, hunched posture, head tilt or twitching).
- Weight loss of up to 20% (typically this is recovered within 7 days).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of animals used within this project (~75%) will experience a moderate level of severity. The remaining animals (~25%) will be expected to be in the mild or subthreshold category.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Although *in vitro* cell culture methods can be used to test the transformative effect of gene mutations on particular cell types, this system cannot recapitulate the complex microenvironment (i.e. the arrangement and physiology of many different types of cells) that exists in tissues and organ systems. Our scientific approach requires the use of cell types that are susceptible to cancer, at specific time points during development and animals remain the most informative model, as they recapitulate the diversity of cell populations, complexity of human development and the crosstalk between tumour and stromal (non-tumour) cells in human cancers. Our careful use of human genomic and in vitro cell culture assays (most notably to test signalling elements and novel therapies) has capacity to triage cancer causing lesions, dramatically reducing the number of animals used in exploratory genetic models and markedly increases the efficiency with which we develop cancer models.

Animals are required to achieve our aim as regulatory and research bodies require preclinical assessment of potential therapies in animal models before their translation to the human clinic. In spite of this we will continue to use cell culture methods, such as drug sensitivity studies, and in vitro

radiation and chemotherapy combination studies to optimise the selection of agents and minimise the numbers of animals used in testing potential therapeutic agents to those that have shown the most promising effects.

Which non-animal alternatives did you consider for use in this project?

In the careful design of our research project, we considered the use of methods that would minimise the need for animals, such as cerebral organoid (3D cultures of mixtures of brain cells of the appropriate type) cultures. However, such systems do not always model the complex nature of the organ development or the biology of cancer.

Where possible we will use alternative research approaches that do not involve the use of animals. In particular we will use in vitro methods, including culture of patient derived cells and the generation of iPSCs (induced pluripotent stem cells), to screen potential therapeutic agents for efficacy against tumour cells.

Why were they not suitable?

Although the use of cell culture methods will enable us to reduce the use of animals, they cannot fully replace them as they do not model the complexity of the organ or the tumour. Organoid, cell culture and iPSC systems lack many of the features that accompany a human brain tumour, such as a functional blood-brain barrier, the diverse cell populations and the immune system. The fact that we require the use of specific cancer susceptible cell types at specific developmental stages and that regulatory bodies require the preclinical assessment of novel therapies in animal models prior to translation to human trials, dictates that we must use animal models to achieve our aims. It is therefore, critical that we use the best models and methods for this research.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our use of *in vitro* (cell culture) methods reduces the number of animals required for the *in vivo* investigation stage of this research. Furthermore, each of our mouse model experiments has a careful statistical design that is aimed at minimising the use of animals while ensuring robust and meaningful statistical end points. These animal numbers are selected in collaboration with highly qualified statisticians and are based on our extensive experience with brain tumour mouse models.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In designing the experiments described within this project we aimed as much as possible to reduce, replace and refine our use of animal models. In order to achieve this, whenever possible, we will use cell culture methods to replace or reduce the number of animals used in studies. For example, in trials of novel drugs, we will first determine how efficient the drug is in cell lines that have previously been generated from an animal model. This will be performed using a series stringent downstream analysis methods. These *in vitro* results will help indicate how and whether a mouse study should be performed.

Where mouse model experiments are required, we have used a careful experimental design that allows us to minimise the use of animals, whilst achieving the maximum amount of data and maintaining reliable statistical end points.

In many of our animal models we have included the use of a bioluminescent reporter (a gene that makes the tumour cells glow) to enable visualisation of the growing tumour or its therapeutic response over time. As a result, we will be able to reduce the number of animals required to achieve both growth and survival data. The bioluminescent information can also be used to determine a point at which a tumour is established but is not large enough to cause symptoms, indicating the correct stage to start treatment studies. This also reduces the severity of the overall experience of the mouse.

We have also worked closely with a senior biostatistician, to develop and implement an experimental design that enables the adjustment of mouse numbers used in a particular group, according to the latest information.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To minimise the number of animals required in our project we ensure the use of the most up to date laboratory methods for processing materials collected from animals used in this licence. By doing this we are often able to achieve the maximum amount of data from smaller samples. In this way we can split samples collected from one mouse into downstream numerous applications. For example, one piece of tissue can be used to generate fresh cells for culture, frozen samples for molecular analysis and fixed material for histology.

By employing standardised SOPs for our experimental design, particularly in our novel treatment studies, we can use data from control animals in one study, as controls in another (within the same tumour type) and thereby reducing the number of animals required in this project. Where needed pilot studies will be performed, which will inform the design of subsequent studies, potentially enabling the reduction of numbers.

Where possible, our genetically engineered mouse strains will be maintained as homozygous in order to reduce the breeding needed to generate mice of the required genotype and to optimise the efficiency of offspring carrying the appropriate alleles for the experimental purposes.

As part of a good standard of practice, each experiment will be preceded by the writing of an experimental study protocol which will outline our objectives and methodology and will include statistical endpoints. All of these will ensure that we are using the optimal number of animals to achieve the aims of this project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are the only species that are employed in our protocols. In particular, we will use genetically engineered mouse models (GEMMs) that replicate three of the most important human paediatric brain tumours; Ependymoma, Choroid Plexus Carcinoma and Medulloblastoma. These strains have been specifically generated to carry the genetic mutations that lead to tumour formation in the human diseases. In this project we will use these strains to explore the biology of the disease by inducing tumour formation using a variety of methods including conventional breeding, *in utero* injection or injection of agents. Animals in these studies will be maintained under normal conditions until a specified time, at which point material will be collected and analysed as previously described. These methods are all designed to result in no more than a temporary discomfort to the mice. These models may further be used in the preclinical assessment of novel treatments, as they most closely recapitulate the human condition.

Orthotopic implantation of tumour or patient derived cells, for use in treatment studies, will be carried out in immune compromised CD1 nude or NOD SCID Gamma mice (NSG). These intracerebral transplantation models are necessary to avoid rejection of human material and to enable rapid tumour growth for treatment studies, as these cells are expected to engraft and induce tumours in transplanted mice. The methods involved in assessing novel treatments can result in a more severe experience for the mice and so to reduce the number of animals enrolled in such studies, we have refined our workflow to include initial testing of leading agents using a subcutaneous implantation model. Subcutaneous tumour models are well documented in literature for use in such studies, but as they lack the blood-brain barrier they cannot be used to replace the need for orthotopic implant models in the final test of a novel therapeutic.

The majority of our mouse models utilise a bioluminescent tag that enables the real time monitoring of tumour development. Using this allows us to limit the suffering for the mice by providing a guide for progression to development of clinical signs, and thereby a humane endpoint by which to ensure suffering is kept to a minimum.

We will minimise suffering by adhering to the best practice guidance, currently the NCRI guidelines for the welfare and use of animals in cancer research. Every protocol proposed in this licence is the most refined for the purpose and designed to cause the minimum distress and suffering to the animals.

Why can’t you use animals that are less sentient?

Mice are the most relevant species with the least sentience that we can use to carry out the research proposed in this project. Their short lifespan (approximately 2 years) allows for studies in which disease

progression can be monitored throughout development to humane endpoint, mimicking far closer the disease progression in humans. Further to this, the short gestation time (~3 weeks), extensive published knowledge and the array of techniques that enable manipulation of the mouse genome, allows us access to genetically modified animals in which we can explore the effects of genes on normal brain development and tumour formation in a species where the process is similar to that in humans. Less sentient species do not follow this same developmental program. Non-animal models cannot recapitulate the complex context of tissues in which cancers develop, particularly in the brain where the blood-brain barrier and functional immune system are key features, or the complexities of the tumours themselves. These features are critical for understanding the impact of potential novel therapeutic approaches.

The advancement of knowledge and development of concepts to improve human and animal health and well-being requires the use of living animals. Exhaustive literature searches in brain tumorigenesis show that our tumour systems are the most accurate models for the study of these diseases. The use of our preclinical mouse hospital in the testing of new treatments includes the use of combinatorial approaches, including irradiation and chemotherapy is critical as it allows the assessment of novel agents in context of treatment actually received in human clinics. This is the best approach to find new therapies that will succeed in the clinic and will further reduce the number of animals needed to achieve clinical impact.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Our procedures will be regularly assessed throughout the project in order to ensure that they are being performed in the most refined manner. Refinements will be routinely made over the course of the project through constant advice from veterinary and husbandry staff, as well as through regular liaison with clinical practitioners. For example, we will work closely with the NVS to look for a less aversive inhalation anaesthesia in order to reduce stress on the animals.

If needed amendments to the licence will be applied for, for example, if new procedures are needed to improve the welfare of animals.

Within our last licence period we have made several refinements to our procedures, in order to ensure that we are using the most refined method for the study and reduce suffering to the mouse. For example:

- We have worked closely with practicing clinical neurosurgeons to refine our surgical procedures, including the use of biopsy scissors in order to resect tumours and a fine cauterisation pen for stopping bleeding following resection. Both of these refinements have resulted in fewer losses.

- The use of osmotic minipumps to deliver chemotherapeutic agents is more invasive, however, by using a pump that can remain in place for one week, we are able to reduce the number of injections that a mouse may receive and thereby reducing handling and stress for the animal. In order to reduce stress on the mouse even further the pump is implanted under anaesthesia and pain relief is provided.
- Finally, the use of oral gavage to deliver tamoxifen to pregnant dams has been shown in the literature to carry a reduced risk of embryo loss.

We aim in this licence to determine treatment regimens that reduce exposure to anaesthesia but that result in a better prognosis for the mice and ultimately for human patients.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Work in this licence will be undertaken in accordance with the principles set out in the Guidelines for the welfare and use of animals in cancer research: British Journal of Cancer (2010):102: 1555-1577 and the guidelines published by the NC3Rs, which are updated regularly with the best current practice.

All surgical procedures will be performed under the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) and blood sampling will be performed as recommended in the NC3Rs guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We currently follow updates provided by the National Centre for the 3Rs through receipt of electronic and printed media and through regular attendance at our Institutes AWERB committee. Throughout this project we will

- communicate constantly with veterinary, NACWOs and husbandry staff,
- receive updates from the NC3Rs and
- will attend NC3R and scientific conferences.

These approaches will allow us to be aware of the most recent advances in the field and will enable us to implement these into our research in a timely manner.

Home Office

NON-TECHNICAL SUMMARY

2.Analysis of cell signalling in a zebrafish disease regeneration model

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

regeneration, stem cells, signal transduction, developmental biology, disease modelling

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand the important roles that cell signalling plays during disease and regeneration. By understanding better how different signalling pathways interact we will be able to inform new clinical approaches.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A firm understanding of how cells communicate in a simple vertebrate will inform clinical approaches to disease and disability. Cell signalling molecules are the master regulators of cell behaviour and cell fate. This has made them popular targets for pharmaceutical strategies and the development of new medicines. A good example of how our work has informed clinical studies is in the understanding of an inherited childhood tumour disease. Our studies have shown how the disease functions in the initiation of cartilage tumours (osteochondromas). This understanding has led to the development of a drug for the treatment to suppress osteochondroma formation. Understanding the important roles that signalling plays in different disease states and during regeneration is our ongoing aim. This project promises to aid in the treatment of many degenerative diseases and in recovery from injury or surgery.

What outputs do you think you will see at the end of this project?

The outputs from this project will include publication of our study in highly respected journals, presentation of results at international scientific conferences and patents based upon our discoveries. In particular we will reveal new signalling pathways that identify and recruit regenerative cells. We will characterise how these pathways affect cell behaviour at the single cell level: How these signals control cell differentiation, cell shape changes, migration and proliferation. We will use genomic approaches to understand how global transcriptional changes affect these processes. All of these finding will enhance our ability to identify new protein targets for pharmaceutical intervention in a clinical setting.

Who or what will benefit from these outputs, and how?

The understanding of how cells communicate underpins much of our understanding of human disease. For example, mutations that affect signalling genes often confer cancer. One such gene is HER2 which when mutated may result in breast cancer. Genetic tests can now identify individuals that are susceptible to breast cancers long before the cancer forms. Herceptin is a commonly used treatment for breast cancers that was developed based upon our understanding of how HER2 acts in cell communication. It is difficult to predict at this point the specific clinical problems that will be impacted by our research as our studies focus on the basic understanding of cellular interactions. It is also difficult to estimate the time it will take for significant clinical milestones to be achieved based upon our basic research findings. However, it is likely that our results will at some point help clinicians to better understand how altered signalling results in different disease states. Many signalling molecules also make excellent targets for pharmaceutical intervention. By identifying new important interactions we will be providing pharmaceutical companies with novel targets for the design of inhibitors. Further beneficiaries of our research are pharmaceutical companies with which we have collaborated in the past. Such collaborations between academia and industrial partners is crucial for scientific, medical

and economic progress to be made. Finally, researchers in the fields of biology and chemistry will understand and act upon our findings in their own research domains.

How will you look to maximise the outputs of this work?

We are a highly collaborative research group with interactions both nationally and internationally. Our group has organised two international research conferences and our results have been published in over 50 research papers.

Species and numbers of animals expected to be used

- Zebra fish: 31,500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Zebrafish was chosen for this study because of its low sentience (neuronal complexity) and because is a vertebrate which has many of the same genes as humans. This project will involve working with all stages of the life cycle. This is because we would like to understand cell behaviour throughout the life course.

Typically, what will be done to an animal used in your project?

Most of the fish for this project will be used for breeding purposes. Typically, these fish mate naturally and lay eggs to establish the next generation. Most of these fish will be transgenic or carry mutations. We will also use fish to generate new transgenic lines. Typically, this involves adding genetic material to the eggs when they are at the one cell stage, then raising the fish normally. These two procedures will account for more than 95% of the animals used in this study. Of the remaining fish they will experience more invasive techniques such as being exposed to pharmaceutical treatments, or having small pieces of their fins removed. These invasive techniques are necessary to understand how cell signalling functions during regeneration.

What are the expected impacts and/or adverse effects for the animals during your project?

Although the vast majority of our animals lead healthy lives, there may be unforeseen effects on the health of the fish due to our experimental regimens. These include the formation of open sores, necrosis, ragged fins, very slow and irregular heartbeat or oedema, tumours, abnormal behaviour and weight loss. Animals that display these signs of ill health will be killed by a Schedule 1 method. Our protocols are designed to minimise suffering and if animals are in poor health or visible pain the experiment will be aborted.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most animals will experience only mild severity. A small proportion (less than 2%) may experience moderate severity.

What will happen to animals at the end of this project?

- Killed
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The study of the role of combinatorial cell signalling involves different tissues and the complex extracellular milieu that is present in living organisms.

Which non-animal alternatives did you consider for use in this project?

Our project builds upon the knowledge gleaned from in vitro studies. As this project develops, we will seek to move experimentation into in vitro systems. We will do this through collaborations with tissue culture laboratories.

Why were they not suitable?

In vitro studies do not allow us to identify new signalling events from unexpected sources. The complex interactions that occur between multiple tissues cannot be recreated in an in vitro system.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This project relies on analysis of mutant and transgenic fish in the heterozygous and homozygous state. Strains are usually maintained by crossing heterozygous carriers to wildtype stock (an outcross). To obtain a reliable and consistent number of fish, one must have a sizeable colony of identified heterozygous carriers. These are usually obtained by random crossing of pairs of fish which means that we can only estimate the number of fish that are needed. Furthermore, sex ratio inequalities make it difficult to know how many fish are needed: often the ratios of male to female are as low as 1:10 in a given tank. Thus to be sure that enough pairs can be tested to obtain enough carriers, a large number of outcrossed fish must be raised. Maintenance of mutant and transgenic strains makes up the majority of the number of fish used for this project licence.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will try to minimise the number of animals used by careful experimental planning, but as these experiments are exploratory in nature it is difficult to precisely predict the number of animals needed for this project. Where applicable, we will write a individual study plan for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, details of the experimental material, and the size of the experiment (number of groups, numbers of fish or embryos/group); and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and with the treatment differences that are to be estimated). When ever possible we will seek to move experimentation into in vitro systems. We will do this through collaborations with tissue culture laboratories.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have a highly successful breeding program that is a model for zebrafish facilities around the world. For experiments, we only perform the necessary number of experiments to obtain publishable (significant) data. Our protocols are designed to minimise suffering and if animals are in poor health or visible pain the experiment will be aborted.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use zebrafish (Danio rerio). Most of the animals used in this project are simply being bred which is a regulated procedure.

As the embryos develop ex utero, experiments and harvesting embryos can be done without harming the parent. Zebrafish are an extremely tractable system and techniques such as transplantation, injection of genetic material and GFP analysis are standard in the laboratory. The transparency of zebrafish allows one to follow the behaviour of labelled cells (i.e. green fluorescent protein) over long periods of time. Chemical modifiers are easily applied by addition to the media. Some of our study involves analysis of regeneration: In our bone crush assay, a single ray in the tail fin is crushed: this damages as area no larger than 0.01mm2 and containing approximately 50 cells. This damage is not visible to the naked eye. Tail excision in adults involves removing 0.2 to 0.6 cm from the tail and is visible. The tail is very thin, and the total weight of the tissue removed is 2-4 milligrams. In comparison a fish weighs 1-2gms. Thus although with adult tail amputation damage is visible, it is still a very small amount when compared to the entire animal. Fish repair damaged fin tissue rapidly and these types of injuries occur regularly in nature.

Why can’t you use animals that are less sentient?

We use zebrafish (Danio rerio) for our research because it is the least sentient vertebrate model system. This model allows us to study complex cell behaviour involving different tissues (e.g. skeletal tissues) in a simple organism. Most of our research is done in immature life stages, however it is sometimes necessary to confirm important results in older life stages to draw more general conclusions.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Procedures that may cause distress to the fish are performed under deep anaesthesia. After procedures fish will be closely observed and monitored for any signs of distress. If a fish is showing signs of distress then the experiment is stopped and the protocol will be reviewed.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

There are many useful resources on the NC3Rs website (e.g. webinar July 2020: “Best practice in experimental design)”. Zebrafish husbandry publications include Zebrafish by Christiane Nusslein-Volhard and Ralf Dahm in Practical Approach Series (OUP) and The zebrafish book: A guide for the laboratory use of zebrafish Danio* (Brachydanio) rerio by Monte Westerfield, Institute of Neuroscience, University of Oregon. The new ARRIVE Guidelines 2020 will also be strictly adhered to (https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000410)

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

NC3Rs regularly holds online workshops and events aimed to improve experimental procedures with a focus on the 3Rs. We continually review and refine our protocols with discussion between our researchers,our NACWOs and our NVS.

Home Office

NON-TECHNICAL SUMMARY

2.Angiogenesis in health and disease

Project duration

1 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

angiogenesis, skeletal muscle, remodelling, sarcopenia, hypertrophy

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We have identified novel approaches to regain control over blood vessel growth that is lost in many diseases, allowing us to better understand the cells and molecules involved in regulation and devise more targeted interventions. Much of this work has been successfully addressed, but Covid-19 lockdown prevented the final experiments needed to complete studies for 2 PhD students, so we wish to have a short-term licence to continue the current licence aims, objectives and procedures.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

An adequate, but not excessive, blood supply is critical to health
. Hence, numerous pathologies arise as a consequence of disrupted vessel growth.

What outputs do you think you will see at the end of this project?

The aims of the work are expected to be realised, given that this is a licence intended to complete the final experiments in a series already started before interruption by Covid-19 lockdown. We will continue to generate outputs aimed at wide dissemination of the results including posters and oral communications at scientific meetings, peer-reviewed articles and reviews, research seminars and public engagement events.

Who or what will benefit from these outputs, and how?

Cell and molecular biologists, physiologists and biomedical scientists will benefit from these outputs in the short term. Public understanding of science is part of the long-term goal. Adoption of the findings may influence clinical practice and aid in rehabilitation medicine.

How will you look to maximise the outputs of this work?

Immediate beneficiaries are other researchers in the field, or other fields of biomedical research; patients with relevant pathologies and/or clinicians attempting treatment; society as a whole due to the social value of new knowledge, which may in time form the basis of economic benefit. Benefits will be utilised to inform better diagnosis and new treatments, or improved quality of life, based on these pre-clinical data; intellectual gain will accrue from dissemination of new knowledge, e.g. publishing in scientific journals and informing other research; these data will complete publications begun under the current PPL. The timeline of benefits will be varied: immediate benefits will be the provision of essential data to inform the next stage of the project; short to medium term benefits will accrue from published

papers to inform the scientific field; long term benefits may include major discoveries based on this original work, but the nature of scientific research cautions against making unrealistic goals on this score. Benefits will be maximised by use of data as the basis for subsequent grants, thereby continuing the process of knowledge expansion, and where possible by exploiting the intellectual property rights in conjunction with the University legal department.

Species and numbers of animals expected to be used

- Mice: 36
- Rats: 48

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using animals previously demonstrated to exhibit similar mechanisms of muscle hypertrophy/atrophy as seen in humans, which will allow us to explore new ways of supporting exercise tolerance in conditions where mobility is limited.

Typically, what will be done to an animal used in your project?

For some animals, they will be put to sleep and one hindlimb muscle removed; this may occur in humans following disease, cancer or a traffic accident.

What are the expected impacts and/or adverse effects for the animals during your project?

We take care to administer pain relief after surgery, and the animals recover very quickly - indeed, they have been observed hanging upside down on wire cage lids within 24 hours, suggesting there are no serious impediments to locomotion and routine activity.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All surgery is classified as being of moderate severity; 20 years experience has suggested little difference in animal response among categories.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

By careful monitoring of outcomes, we have managed to reduce the duration and intensity of muscle activity required to elicit vessel growth, have refined the interventions to reduce the discomfort, but as we are investigating a complex integrated response have not yet found a suitable alternative to use of whole animal models. As this project seeks to complete two PhD studies interrupted by Covid-19, it would be inappropriate to adjust any procedures at this stage.

Which non-animal alternatives did you consider for use in this project?

N/A

Why were they not suitable?

Biology is inherently complex, and development of sustainable therapy cannot rely on simplistic models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have many years experience using these animals with these or similar interventions, and seek to use the minimum number that will provide robust data: ambiguous findings arising from the use of inadequate sample size to allow appropriate statistical analysis would be a waste of animals.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have previously engaged the services of biostatisticians to ensure were using adequate, but not excessive numbers of animals. Our long experience in conducting such experiments has demonstrated the validity of this approach, in that our findings have been replicated by other laboratories around the world.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We always attempt to derive as much useful data from each animal, to both maximise the benefit derived from each animal used but also to minimise the use of additional animals. This may take the form of tissue samples used for two different analyses, or measurements made under terminal anaesthesia followed by ex vivo experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use a model of inducing muscle hypertrophy that is consistent with the extent of compensatory growth seen in a number of clinical conditions, without inducing pathological responses as seen in some alternative methods. For development of obesity and complications we use a strain of rat that spontaneously develops this condition.

Why can’t you use animals that are less sentient?

We have demonstrated over a number of years that the biological responses we are studying are conserved among mammals, but there is little evidence to suggest less sentient animals would have the same response. As the therapeutic goals are aimed at muscle dysfunction most typically experienced in adult life, using immature stages would be inappropriate as we know there are mechanisms aiding recovery that are lost with age. The response we are studying are part of a dynamic remodelling process, and hence require chronic intervention, beyond the scope of anaesthetic insensitivity.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

This is the final stage in an established research program, so further adjustment to procedure would invalidate much of the data generated from animals used so far, so would violate our commitment to upholding the 3Rs principles.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow current advice from the Home Office regarding regulatory procedures and advice, and follow current standards laid out by reputable biomedical science journals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We subscribe to relevant sources of reliable animal welfare advances, such as Understanding Animal Research and the National Centre for the replacement, Refinement & Reduction of Animals in Research, and communication with local named veterinary surgeon and animal care staff.

Home Office

NON-TECHNICAL SUMMARY

3.Antibody-based therapeutics for cancer

Project duration

5 years 0 months

Project purpose

- (a) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (b) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to develop new medicines that modulate the body's own response to cancer to provide better and safer treatments.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Although in recent decades a lot of progress have been made in prevention, diagnosis, and treatment of cancer, it is still the second leading cause of death globally, accounting for an estimated 9.6 million deaths in 2018. In the same year, approximately 17 million new cancer cases were reported. Due to the aging population, genetic factors, and unhealthy life-style choices, the cancer burden continues to grow globally. This exerts a tremendous physical, emotional and financial strain not only on individuals and their families, but also on communities and national health systems.

Currently available cancer treatment options are often invasive, painful and damaging to the patient, these include surgery, chemotherapy, and radiotherapy. In recent years, a novel class of therapeutics which directly engage and modulate patient's immune system to fight cancer has been introduced to the clinic. These new drugs, predominantly antibodies, have shown great potential in a percentage of patients with particular types of cancer and for whom other therapeutic options had been exhausted. However, these new drugs have failed to change the overall outlook in which only half of cancer patients will survive for 10 years or more after cancer diagnosis. It is important that new medicines are found that can treat patients with cancer, without causing further harm.

What outputs do you think you will see at the end of this project?

The main outputs of this project licence will be the development of novel therapeutics for the treatment of cancer.

One of our antibody is currently being tested in clinical trials and another is in advanced stages of pre-clinical development. The studies performed within this project will help us to define which diseases can be targeted with these drugs, which dosage is the most indicated, and what other drugs could be used in combinations to have a stronger anti-tumour effect.

For our programs that are at earlier phases of development, we will gain the necessary knowledge to validate their targets and potentially obtain antibodies or antibody-based therapeutics that could progressed to clinical development.

Who or what will benefit from these outputs, and how?

At the moment, many cancer patients have a poor prognosis and their disease continues to progress despite being treated with current medicines. This project will provide the supporting data to develop novel therapeutic antibodies that will benefit patients either as stand-alone therapies or in combination with other agents, increasing response rates and ultimately being able to bring about effective disease control in a greater number of patients. This would benefit not only individual patients, but also communities and national health systems.

How will you look to maximise the outputs of this work?

Our findings will be presented at national and international scientific conferences and meeting as posters and talks, and eventually published as scientific articles and patents.

The results of the accompanying clinical trials will be included in the entries to the ClinicalTrials.gov and/or published in the scientific journals.

Species and numbers of animals expected to be used

- Mice: 12500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In this project, we are developing antibody-based therapeutics against cancer.

Mice are used in oncology studies because of well understood genetics, availability of well validated tumour models, and relative ease of manipulation of the immune system. As tumour growth is a relatively long process, we will be using young but fully adult mice (6-12 weeks old) so that their immune system is fully matured and can resemble the human situation.

Typically, what will be done to an animal used in your project?

In this project, we will be administering therapeutic agents to mice challenged with tumours, using predominantly subcutaneous (implanted under the skin) and occasionally orthotopic (implanted to relevant organs) tumour models. In rare occasions, when needed for the subsequent steps, mice could be pre-treated before implantation of the tumour.

When using subcutaneous tumour models, mice will be tagged usually by the implantation of a chip for identification purposes, then partly shaved on the site of injection and injected with cancer cells or fragments under brief anaesthesia. After recovery, tumours will be allowed to grow while the mice are being monitored. When the tumour is established, usually by 6-15 days, treatment will commence. In most cases mice will be dosed with intraperitoneal injection (through the wall of the abdominal cavity) 2-3 times a week for up to 3 weeks. On some occasions, treatments may be administered more often and via other routes e.g. orally or intravenously. During the study mice will be observed, weighed and tumour size measured non-invasively usually 3 times a week. The studies will be terminated when enough data on the anti-tumour effects of the treatments have been collected and no additional scientific knowledge could be obtained, usually no more than 5-6 weeks after tumour implantation.

When using orthotopic tumour models, the tumour cells will be implanted surgically into the relevant organs, e.g. liver, kidney capsule, or pancreas, under anaesthesia. The surgical wound will be repaired by suturing and mice will be allowed to recover from anaesthesia under observation. After that, mice will be monitored, weighed, and the tumour growth estimated by palpation. When tumours are established (depending on the model, between 5-20 days after tumour implantation), mice will be treated with antibodies, cells and/or other compounds, and monitored as described for the subcutaneous models, with the exception of tumour growth being estimated/measured by palpation.

What are the expected impacts and/or adverse effects for the animals during your project?

Most of the animals used in this project will be bearing a subcutaneous tumour and will be dosed with antibody-based treatments. Growth of tumours implanted subcutaneously are expected to have only mild adverse effects, unless it undergoes ulceration or necrosis (death of tissues), with the latter expected only in rare cases. Ulcerations will be graded and closely monitored; mice will be humanely killed within 48 hours from the appearance of a grade 3 wound (a full thickness skin loss with shallow tissue damage).

Physiological responses to the treatments such as tumour growth and antibody mediated biological activities may result in some adverse effects. Antibodies are usually very well tolerated, but their administration could lead to temporary mild adverse effects such as weight loss or piloerection. These effects usually resolve within 2 days from appearance.

Orthotopic implantation of cells might results in temporary mild pain as in most cases it requires surgery and suturing; these will however be ameliorated by the administration of anti-inflammatory drugs and analgesics. Orthotopic tumours may in some cases metastasize more easily to other sites causing behavioural changes and weight loss that will be regularly monitored and alleviate whenever possible, for example with provision of food supplement on the cage floor.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In this project, individual procedures are expected to result in only mild adverse effects in most mice. However, most experiments will include more than one treatment/procedure which will result in a cumulative level of severity likely to be moderate for all the animals used in Protocols 1, 2 and 3.

Most mice used under Protocol 4 will experience procedures only under terminal anaesthesia, thus they are expected to be classified as non-recovery.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Cancer cells co-opt many immune cells, including macrophages, neutrophils, T-cells and non-immune supportive cells such as fibroblasts or endothelial cells that build blood vessels, to suppress the immune system and create an environment which supports the growth of tumours. In this project, we will be developing antibody-based therapeutics that re-invigorates immune cells in the tumour micro-environment so that they can fight cancer.

The intricate interaction between cancer cells and the immune system within the tumour, but also systemically, can only be modelled properly in an animal setting.

Which non-animal alternatives did you consider for use in this project?

It is possible to model some properties of the tumour micro-environment in 3D cell cultures such as spheroids or organoids. They are usually very simple and composed of a single cell type derived from human or animal tumours. They allow for medium throughput screening of drugs that directly affect growth of tumour cells.

It is also possible to study cancer in human tumour explant cultures. These are derived from the patient resections and are the best representation of human disease tissue. They are, however, very difficult to obtain, the amount of tissue is generally small thus allows only to study a limited number of conditions/treatments.

Why were they not suitable?

3D culture systems are very simplistic. In most cases, they include only single cell type (cancer cells) and as our test molecules will be targeting immune cells, they cannot be used directly in this model. We and others are however, developing 3D culture systems that in future might include more cell types including at least some immune cells.

Patients explants are complex models, which however cannot be maintained in culture for more than just a few days. Some aspects of anti-cancer immune responses often take a week or more to fully develop and therefore cannot be studied in this system.

Both models also lack interactions with other immune system organs such as lymph nodes, bone marrow, and spleen that play an important role in anti-tumour immunity.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We are currently developing a similar number of oncology drug candidates as during the course of 2017-2018, when around 60 studies a year were performed under the previous licence, using an average of 2500 mice a year. We foresee a requirement for a similar number of animals being needed to support these programs.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To ensure we use the minimum number of animals, Experiments will be carefully designed, taking into account the expected outcomes and statistical analysis of the data. Power calculations will be performed using the NC3R's Experimental Design Assistant, Gpower3.0 or other relevant software. Data previously generated in-house and literature searches, together with pilot experiments, will help to have preliminary information that will be used in the experimental design.

Sources of variability will be controlled whenever possible (cage identity, sex, age, operators etc.). Animal assignment to experimental groups will be randomized and experiments will be performed on comparable numbers of male and female mice to account for possible sex bias in the responses. If there is a known gender difference in the response to the model then the appropriate gender for the type of study will be used.

For each model, the incidence of the tumour uptake can affect the number of mice used per group. Whenever possible, we will use models with an incidence close to 100%, using low incidence models only when no alternative can be found or there is scientific evidence that these are the best models to study a specific mechanism.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

All our antibodies and related molecules undergo extensive characterization of their activities in a suite of cellular and tissue assays in vitro. Only a small number of selected candidate molecules (usually from 2 to 5) is used in animal experimentation.

To further reduce the number of used mice, we will maximise the amount of data obtainable from each single experiment. Whenever possible, for example, we will obtain preliminary information on the levels of the drug in the mouse blood post injection while testing its general tolerability. This may allow us to identify any dose-related issues with a molecule and help us to optimize the dosing regimens. Additionally, we may collect tissues at the end of the experiment and test bio-distribution of our compounds and possible pharmacodynamic effects on mouse physiology.

We have also recently introduced a micro-sampling method for blood collection from tail vein for plasma isolation. Using this method, we can collect blood from the same animals over multiple time points and generate data using a reduced number of animals compared to standard blood collection methods. Following this refinement, we were able to reduce the number of mice used in this protocol by half. We will also optimize the tumour biopsy sampling technique, to reduce the number of mice needed to analyse the tumour micro-environment.

Additionally, collecting mouse tissues and performing *ex vivo* experiments will allow us to further reduce the number of live animals used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mouse models of cancer have been widely used in the development of our understanding of tumour immunology and have successfully predicted the mechanism of action and the therapeutic utility of new medicines. There is a large body of literature from which we can source information to help us define

the most efficient means of obtaining scientific data from these models while causing the least harm to the animals.

Why can’t you use animals that are less sentient?

In this project, we will investigate therapeutic interventions that modulate the immune system to fight cancer. Many of the targets for these interventions (both particular cell types and proteins) are not present in non-mammalian species. For this reason, mice with their very well understood genetics and immunology, plethora of well-established tumour models, and developed technologies to manipulate their genome are currently the species of choice for oncology research.

As tumour growth is a relatively long process, we will be using young but fully adult mice (6-12 weeks old) so that their immune system is fully matured and can resemble the human situation.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Mice will be housed in a state-of-the-art animal unit where a high standard of environmental enrichment is provided to animals. Animals will be housed in groups to allow for social interaction. However, when required for animal husbandry purposes, they might be housed singly but not for longer than 8 weeks and enhanced environmental enrichment will be provided.

When appropriate, suitable analgesia and anaesthetic regimes will be used to minimize animals suffering. Where novel agents are administered, the duration and frequency of monitoring will be increased to ensure that no animal will suffer unduly. Humane endpoints will be continuously evaluated for each model and refined wherever feasible.

All studies will be reviewed internally by the Project Licence holder and the personal licence holders involved before commencing to ensure that experiments are aligned with the Licence and reflect the implementation of best practices.

All the compounds used in animal studies will undergo extensive quality control checks and where possible, we will use drugs sourced at clinical grade. Tumour cell-lines will be free from infectious contaminants, confirmed by external tests for various rodent pathogens.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The "Guidelines for the welfare and use of animals in cancer research" published in the British Journal of Cancer 2010, the PREPARE guidelines and our Campus Animal Usage guidelines will all be used to assist with the planning and of experiments. The “Guiding Principles for preparing for and undertaking aseptic surgery” (LASA 2010) guidelines will be followed for surgical procedures.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

All staff involved in the design and implementation of animal experiments in line with this project licence will be encouraged to participate in relevant tumour modelling conferences, courses and workshops to stay on top of current trends in the field.

Additionally, they will register on the NC3R mailing list to ensure communication of best practices. After action reviews will occur internally and in consultation with the named people to ensure best practice is implemented at all times.

Home Office

NON-TECHNICAL SUMMARY

5. Arabian killifish; a novel animal model for investigation of embryo development and infection

Project duration

5 years 0 months

Project purpose

(a) Basic research

Key words

Arabian killifish, zebrafish, transgenic fish, mutant, CRISPR/Cas9

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult, pregnant
Arabian killifish, Aphanius dispar	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Zebrafish are currently used as models for embryo development and infection studies. Arabian killifish have characteristics with potential to make them a better model for these studies. The aim of this project is to produce genetically altered zebrafish and Arabian killifish from which naturally spawned eggs and embryos will be collected. These will be used for development and infection studies to compare the two models.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The zebrafish embryo has been a very important model organism for studying development and infection because of the large fecundity and accessibility for high resolution microscopic imaging due to its small size and transparency. However the zebrafish embryos start moving after 15 hours, therefore to trace the cellular and tissue events in the embryo in later embryonic stages, it requires anaesthetics and in such condition, physiological responses are often compromised. On the other hand, the Arabian killifish embryos do not move for the first 4 days therefore it is more suitable for longer tracing of the cell and tissue behaviour. In addition, the yolk of the Arabian killifish is far clearer than the one in zebrafish facilitating higher resolution imaging in many tissues. In case when we use the model for infection studies using human pathogens (e.g. Candida albicans), it is ideal to infection at human body temperature, however zebrafish embryos cannot survive this temperature. We found that the Arabian killifish embryos can survive and develop normally to the hatching stage at 37'C making the model more suitable for studying the behaviour of human pathogens. To establish the Arabian killifish model and to clarify common and distinct features of its cell behaviour and imaging capability, it is necessary to create similar GA animals in two species and compare the performance as model systems. For infection experiments, we will focus on human opportunistic pathogen, Candida albicans. C. albicans normally live in the human body without harming the host. However, when the host health condition is compromised, such as during immunosuppressive treatments, C. albicans is no longer suppressed by immune cells and becomes a life-threatening pathogen. However, the invasive process by C. albicans and its interaction with cells at diverse body sites is not well understood due to the difficulty of imaging infection in vivo. In this project we will modify the activity of the host immune system using our model species and pinpoint the key interactions that make host tissues able to resist or become susceptible to colonisation by C. albicans.

What outputs do you think you will see at the end of this project?

(c) Publications: we will published a few high impact papers reporting importance of our new model system, Arabian killifish, as a model for infection at human body temperature. These papers will include: a description of normal development of the Arabian killifish embryo, technical papers reporting

the method for infection experiments using Arabian killifish, and the molecular mechanisms of infection by Candida albicans and the host response in the Arabian killifish.

(d) Transgenic fish resource: We will establish useful transgenic fish lines in the Arabian killifish. These fish embryos grow with all-over fluorescence or in specific cell-types within the embryo (e.g. immune cells, muscle or brain) therefore the shape, position and size of the body and tissues can be easily visualised under fluorescence microscope. These fish lines will be available for researchers all over the world. The zebrafish is currently a very popular model animal for testing infection and drug screening in both academic institutions and in pharmaceutical industry. As the Arabian killifish has similar advantage for imaging and high through put drug screening, and it can be applied with human body temperature, this system would provide an alternative tool for basic research and drug screening in the research community.

Who or what will benefit from these outputs, and how?

5. Academic researchers: They will have the benefit of using a novel infection tool which is applicable for in vivo live imaging at human body temperature. The researchers who use mice as a model due to the right temperature and many other similarities to human infection will be interested in this model as a replacement system, as this model can do similar temperature experiment in a transparent embryo that provides real-time visual information of infection. Zebrafish researchers will also be interested as this fish permits all experiments that zebrafish can, and it can be done at 37'C. The first few papers will be published in the first 2 years. Then our TG fish lines will become publicly available. Therefore by the end of the 5-year license, many researchers will be using our fish as a model system.

6. Pharmaceutical industry: This model can be used for drug screening and testing. This industry currently uses mice, zebrafish and cell culture systems all of which have disadvantages compared to the Killifish model. We aim to discuss collaborations for its use as a drug-screening system with the aim of starting such research within the 5-year period.

How will you look to maximise the outputs of this work?

(1) Publication: We aim to publish the Arabian killifish model system for Candida infection in a high impact journal to advertise the system to relevant research community. We aim to advertise the system in conference and media as well.

(2) Collaboration with other academic groups: We aim to establish a few collaborations with other research groups and increase the user of the Arabian killifish infection model.

(3) Collaboration with industry: We will seek for collaboration with industry and apply the Arabian killifish system for drug testing and screening of anti-fungus.

Species and numbers of animals expected to be used

Zebra fish: 5000
Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Zebrafish: We will use zebrafish embryos because they are small and transparent therefore are suitable for microscopic observation. Among fish species, zebrafish is the best fish for our study because important tools and protocols are most enriched than for any other fish species. These tools include transgenic fish, mutant fish, genes, antibodies and associated protocols.

Arabian killifish: We will also use the Arabian killifish embryos to complement our study on zebrafish. Though zebrafish is the most suitable model fish for studying infection in developing embryos, they cannot survive at human body temperature. Therefore, for conducting essential experiments that might be affected by temperature, we will use the Arabian killifish as another model fish.

The life stage: We will keep all life stage of the fish just for maintaining the fish in the aquarium. However, when we conduct experiments for collecting data, we will only use the embryonic stages (fertilisation to the stage when the fish start independent feeding). All of these embryos used for experiments will be terminated before independent feeding. The time of independent feeding is 5dpf in zebrafish. But the time in the Arabian killifish can vary between 10 and 12 days depending on the temperature (28 to 37'C). Therefore with 28'C, the latest date of termination is 12dpf and with 30'C or above, the latest date will be 10dpf.

Typically, what will be done to an animal used in your project?

We will collect eggs from the zebrafish and the Arabian killifish from natural spawning. These eggs will be injected with DNA or RNA to create genetically altered fish (F0 generation). These fish will be raised to adult. To select the founder fish that are indeed genetically altered, we will collect eggs from natural spawning and examine either fluorescence under microscope or presence of DNA sequence by embryo PCR. The fish identified as genetically altered will be maintained in the aquarium. These fish will lay eggs by natural spawning. We will collect these eggs, raise them to embryonic stages (stages from fertilisation to the independent feeding) and use these for our experiments. These experiments include general characterisation of the embryo development and injection of a Candida albicans and observe the process of infection and reaction of the host cells (e.g., immune cells) under microscope. All experiments will be finished before independent feeding and the embryos will be all terminated before independent feeding.

What are the expected impacts and/or adverse effects for the animals during your project?

Injected constructs may cause death or developmental abnormalities before fish reach the stage of protection. Harmful genetic alterations in embryos may be evident as altered morphology prior to hatching.

Some genetic alterations may result in a harmful phenotype during post-hatching development, observed as; failure of larvae to inflate their swim bladder, difficulty swimming, altered morphology, abnormal behaviour. Any larval fish showing any of these signs will be euthanised immediately at pre-independent-feeding stage.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Expected severity in these animals would be mild. Injected embryos may show some morphological abnormalities and these will be terminated at embryonic stages. Only healthy embryos will be raised to adult. The transgenes that we use are considered as not harmful. Therefore we consider the process to generate and maintain these GA animals have not more than mild severity.

What will happen to animals at the end of this project?

Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Unless we use animals we cannot obtain a realistic understanding of cell and tissue behaviour. Alternative methods would be cell culture methods, but such methods use one or limited number of cell types and do not reflect the true, complex in vivo environment. In real fish embryos, all key tissues exist and interact with each other in a spatio-temporal manner. In this living environment, realistic alteration of the cell and tissue behaviour in an infected or genetically altered condition will become visible. Such information is indispensable for us to learn the important process of cell dynamics in normal and infected cells, tissues and embryos.

Which non-animal alternatives did you consider for use in this project?

We could consider cell culture systems. Cultured cells (e.g. human or mouse fibroblast cells, macrophage or neutrophil cell lines) would provide host cell response to pathogens such as Candida albicans. Therefore it can be useful for learning cellular response to infection.

Why were they not suitable?

Cell culture method would not allow us to see a true in vivo response to infection, including cooperative action of different type of immune cells, skin cells, neurons, livers and blood cells. Such complex response is only seen in the in vivo system such as fish embryos. Therefore such alternative system is not suitable for us to learn realistic host-pathogen interaction.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Zebrafish: We will keep about 100 fish/line with 10 lines (total 1000 fish), and make new generation every year for 5 years making the total number roughly to 5000 fish.

Arabian killifish: Same as above.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We are highly specialised in generating GA fish lines. With our hand, we can minimise the fish to raise for making new GA fish (100 fish/line).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We aim to further improve efficiency of breeding. Currently we obtain about 30 eggs from each Arabian killifish tank containing about 30 fish. It might be possible that they may lay more eggs if we could provide a better spawning chamber and modifying feeding protocol. We will try such pilot studies using the WT strain. As for zebrafish, the protocol is highly established therefore further improvement of breeding is highly challenging.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Animal models: Zebrafish and Arabian killifish.

Methods: Generating GA fish lines by microinjection, maintain the line and collecting eggs/embryos from natural breeding. These are all highly established and refined methods. In zebrafish, these methods are already established all over the world. In the Arabian killifish, the fish is similar to medaka killifish (ricefish) therefore similar protocol for microinjection is applicable and confirmed as very efficient (not causing adverse effects and can generate GA animal with high efficiency).

Our new model, Arabian killifish, has a strong potential to contribute in refinement of the imaging method. In zebrafish, embryos start to move at 15hpf onward therefore it requires anaesthesia for live imaging. However Arabian killifish embryos do not move for the first 4 to 5 days therefore imaging can be achieved without anaesthesia. This provides a longer time span for imaging without causing any potential harm by anaesthesia.

Why can’t you use animals that are less sentient?

We use fish embryos for experiments because they are vertebrate models which have similar tissues and body pattern to human. But they are also small and transparent allowing live microscopic observation.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will keep updated novel technologies for microinjection, CRISPR/Cas9 and transgenesis from literature and conferences. This may further improve efficiency to generate GA animals (meaning that we might not need to raise 100 founder fish/line in future). We will also be updated in fish husbandry information and technology including maintenance of water quality, food quality, feeding scheme, detection of health issues, population density and enrichment of the aquarium.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow the latest scientific papers and presentations from conferences for generating and maintenance of the GA fish.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will follow NC3Rs webpage, attend 3Rs conference and follow relevant publications.

Home Office

NON-TECHNICAL SUMMARY

6.Assessing metabolic effects of diabetes and metabolic disorders in the heart and other target organs

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Heart, Metabolism, diabetes, magnetic resonance imaging, stem cells

Animal types	Life stages
Mice	adult, juvenile
Rats	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand the interplay between metabolic processes and tissue and organ function in health and disease, with a particular focus on how the development of diabetes affects liver and cardiac function. Damage to a tissue or organ can impair metabolic energy generation and vice versa. By understanding this interplay, we can develop therapeutic approaches to minimise or correct the damage caused by dysfunctional metabolic processes. As part of trying to understand these processes, we will develop new methods for non-invasively imaging them via MRI.

A retrospective assessment of these aims will be due by 03 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Metabolism underpins the function of all organs of the body and generates the energy needed for the heart and other organs to function. The heart is critical to maintaining the supply of blood and oxygen to the body, so that damage to the heart muscle, caused by a heart attack or an increased workload due to high blood pressure, reduces blood flow and energy generation in other organs. These effects are part of the reason why cardiovascular disease is responsible for 27% of all UK deaths. In the UK there are more than 100,000 hospital admissions each year due to heart attack resulting from a blocked artery. This means that part of the heart muscle is not fed with the oxygen and nutrients that it needs, heart cells die and that part of the heart muscle is replaced by a scarred region that doesn't beat properly. The rest of the heart therefore has to work harder to compensate. Recent developments in clinical practice have meant that many more people now survive a heart attack, which is either treated surgically (via stenting to open the blocked artery) or with drugs, but in some cases the damage to the heart is so severe that the heart no longer functions well enough. Currently over 900,000 people in the UK are living with heart failure. By understanding what causes the heart muscle to die and how we can prevent or reduce the damage, we can increase survival and reduce the number of people for whom the damage is so great that the heart begins to fail. Additionally, sometimes patients who are admitted with a heart attack have received damage that is unlikely to recover if treated surgically, but are operated

upon nevertheless. This exposes them to an unnecessary risk of life-changing or life-ending complications, such as stroke, ventricular fibrillation, or cardiac rupture, and it is often usually only realised in the surgical setting of the catheter laboratory that there is nothing that can be done. We are investigating the use of advanced MRI methods with injected compounds to determine whether or not the heart tissue at risk is still viable for surgery, or if it is dead and will not respond to surgical treatment. Also, by identifying exactly which areas of the heart require treatment, we are also investigating whether we can rescue the damaged region of the heart by treatment with stem cells, either injected directly into the heart or attached across the scar to form new viable tissue.

Damage to organs such as the liver and pancreas can alter the supply of sugar and fat in the blood and mean that the heart and other organs cannot generate sufficient energy to work efficiently. By far the most prevalent metabolic disease is diabetes, and its precursor known as metabolic syndrome. There are over 3.5 million people diagnosed with diabetes in the UK and this is estimated to rise to over 5 million by 2025. Worldwide it is estimated to affect 8.5% of the global population. The most common two forms of diabetes are type 1 and type 2 diabetes, of which 90% of people with diabetes have type 2 diabetes. Both type 1 and type 2 diabetes are associated with elevated levels of sugar in the blood and this, over time, leads to complications including vascular problems, high blood pressure and high cholesterol. These are major causes of coronary heart disease, which is recognized to be the cause of death for 80% of people with diabetes. Yet it remains difficult to exactly assess the degree of damage to the heart in diabetic patients, and to understand, non-invasively, what damage has occurred, or is at risk of occurring, or could be prevented in an individual patient. Metabolic syndrome and type 2 diabetes are associated with obesity, which is becoming increasingly prevalent, and which is also linked with sleep apnea, a common breathing disorder that affects many people whilst they sleep. It is thought to affect 2-4% of the population and, in turn, has been shown to increase the risk and severity of type 2 diabetes independent of age and obesity. In diabetes, there is a mismatch between supply of fat and sugar to the heart and other organs, and the ability of those tissues to take up and use those fuels. This results in reduced tissue function and the build-up of unwanted and potentially toxic by-products. We are investigating why this happens and whether we can use drug molecules to restore a more normal metabolism, thereby preventing the development of further organ dysfunction.

What outputs do you think you will see at the end of this project?

Our research will provide valuable insights into the mechanisms behind damage caused by metabolic abnormalities. It will also develop new techniques for imaging this damage, these abnormalities, and their response to treatment. Our work will be published in peer-reviewed journals and presented at national and international conferences. New MR technology and techniques will be shared with other users in the field, in publications and at conferences and workshops, and translated to clinical MRI scanners in the local hospital.
The co-PIs have published over 200 papers which have been cited over 5000 times. One PI is part of the organising committee of our Metabolic Health network which promotes dissemination of research between clinical and pre-clinical departments. She is also an active member of Diabetes UK and is able to discuss her work with the clinical community and patient groups at the annual meeting of the society. Another PI is part of the BHF-funded Regenerative Medicine Network and her work is presented to the BHF community at their annual meetings. The third PI is an active member of the International Society for Magnetic Research in Medicine and his team regularly present at meetings of the society. All PIs are members of the British Society for Cardiovascular Research and encourage members of the group to present their results at the annual meetings of the Society. One PI works in the MR using at the local Hospital and so developments in MR imaging will be directly translated into

the clinic, which we anticipate could be done within 12-18 months.

Where we are exploring pharmacological therapies, we look first to see whether we can repurpose an existing drug to minimise adverse effects and so that potential therapeutic benefits can be rolled out more rapidly. For example, we are studying the effect on the diabetic heart of a drug which currently going through phase 3 clinical trials for anaemia and which could be directly repurposed for treating diabetic cardiomyopathy. Similarly, we have shown that daily treatment with a dietary supplement increased glucose oxidation in the diabetic rat heart. Where there are no existing drugs, we work in collaboration with colleagues in the Department of Chemistry, whose work focuses on the discovery of new drug targets and mechanisms, and the translation of these findings into new clinical candidates. We have clinical Fellows within our research team who undertake clinical studies linked to our preclinical work.

Our collaborator in the Department of Materials has an excellent track record in product commercialisation and has won awards for his innovative products. He is thus ideally placed to take forward successful tissue engineered scaffolds.

Who or what will benefit from these outputs, and how?

The immediate impact will be the sharing of new knowledge and techniques with the scientific research community. This will stem from presentation at conferences worldwide and the high impact publications that will come out during the project duration. Previously our research has been published in the national press, and future work will be promoted in a similar way, so that the public will be aware of our work, and an increased understanding of the links between diet, diabetes and heart health may influence lifestyle choices. Research into the effects of particular diets, such as a high fat diet or a ketogenic diet, could result in changes to clinical advice within the lifetime of this licence.

The design of new MR imaging techniques and hardware will be able to be put into clinical use immediately in the MR research unit at the local hospital. Whereas MR imaging is used routinely in the clinic, MR spectroscopy is still largely a research tool. Research into the effects of re-purposing drugs for improving cardiac function in diabetic patients or following a heart attack would be able to go directly to phase 2 clinical trial, since any adverse effects of these drugs would already be known. Nevertheless, it would take around five years for a drug to be validated through phase 2 and 3 clinical trials. For novel drug compounds, testing in animal models, determining appropriate dosage levels and safety testing in a phase 1 clinical trial would add a further five years to the process. Work in the BHF-funded regenerative medicine network includes feasibility studies in large animal models, so that advances in stem cell therapy could be tested by collaborators within the network. Unlike novel drugs, stem cells do not need to be tested for toxicity and so advances in stem cell therapy could advance to phase 2 clinical trials in the same way as repurposed drugs.

How will you look to maximise the outputs of this work?

We work in close collaboration with other research groups in the field and share our knowledge through Diabetes UK, the British Heart Foundation Regenerative Medicine Network, the International Society of Magnetic Research in Medicine and the British Society for Cardiovascular Research. It is difficult to publish unsuccessful approaches but new data archives such as Data Brief are making this more feasible.

We have collaborations with companies which provide hardware for imaging scanners and with the diabetes research company Novo Nordisk which funds research and Fellowships. From time to time we have students from the Doctoral Training Centres which have links to the pharmaceutical industry.

Species and numbers of animals expected to be used

- Mice: 1700
- Rats: 3350

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

As metabolism and energy generation are linked to the function of the organs of the body, we need to study these effects in a living animal. In order to study how damage to the heart can change the way the blood and oxygen are delivered to the rest of the body, or how metabolic diseases such as diabetes can stop the heart working efficiently, we need to use rodent models which have a four chambered heart and respond to changes in metabolism in a similar way to that in humans. The bulk of our work is done in young adult animals. However, for development of deteriorating organ function and/or metabolism, animals may need to be kept for sufficient time to allow the changes to develop. In some cases we may study juvenile animals, for example to investigate the effects of the onset of diabetes in young adults. We will use genetically altered animals which have a mutation which causes a change to the way the body metabolises fats and sugars. For example, we use animals with a mutation in the leptin receptor so that they continue to eat even when they don't need to and become obese and diabetic. We also use an animal which is used to model muscular dystrophy, as we have found these animals don't respond to insulin as they should.

Typically, what will be done to an animal used in your project?

A wide range of experiments are described in this project. The majority of experiments will be performed in wild-type rodents although we may use genetically altered animals where we want to investigate the role of one particular gene or pathway in the way the body handles fat and carbohydrates or in the development of diabetes or of heart failure.

Protocol 1: In most cases, animals will undergo terminal anaesthesia for removal of the heart or other organs while the heart is still beating. This will ensure that organ function, metabolic proteins and circulating metabolites are representative of the levels *in vivo* as many of these deteriorate very rapidly when the heart stops beating. Animals may be fasted overnight, prior to anaesthesia, as this results in more consistent data since there is no risk of one animal having eaten more recently than other. In some cases, before it is killed, the animal will be given an injection of a substance such as insulin which will cause a change in the way the body handles fats and carbohydrates. Tissue from these animals will be used for *ex vivo* experiments such as a) whole organ perfusion to measure the rate of uptake and metabolism of fats and carbohydrates, b) measurement of oxygen consumption by isolated cells or c) molecular biology to measure levels of genes and proteins.

Protocol 2. A lot of our *in vivo* work involves imaging or spectroscopy to measure function and metabolism of the heart and liver. The early stages of the process development are done using ‘phantoms’ designed to mimic live tissue. However, we need to image live animals where we are measuring heart function so the final stages of method development will use animals. These may be imaged on several occasions but generally no more than once or twice per week and no more than 10 times in total. These animals may have an injection under anaesthesia to deliver contrast agents or drugs to increase the heart rate temporarily. Under this protocol we may image genetically altered animals which are predicted to have problems with heart or liver function as a result of, or resulting in, impaired metabolism.

Protocols 3 and 6. As diabetes is a metabolic disease, we are particularly interested in the changes induced by obesity and diabetes at various stages of the disease. Diabetes can be induced in animals using a compound called streptozotocin (STZ) which kills some or all of the insulin-producing cells in

the pancreas. To induce type 2 diabetes, animals are fed a high fat diet for 3 weeks and then have a low dose of STZ. This model mimics the early stages of the disease as these animals become obese and show increased levels of glucose and insulin in the blood. To induce type 1 diabetes we give a higher dose of STZ which kills the majority of insulin-producing cells so these animals have very high levels of glucose and low plasma insulin. We may also use genetically altered animals which become diabetic where these give a more appropriate phenotype for the question we are asking. The animals will have blood samples taken at intervals to determine development of the disease and may be imaged using techniques developed in protocol 2. Some animals will be given a drug in their food or drink or, if necessary, by injection to establish whether this changes the disease progression. At the end of the experiment we will take tissue for *ex vivo* experiments as in protocol 1.

We also use animals with diabetes on protocol 4 as we know that diabetic patients are at greater risk of heart failure after a heart attack and we are trying to establish why this happens and how to prevent it. In these animals, we will ensure that the diabetes is established and stable before proceeding to further experiments.

Protocols 4 is a surgical protocol designed to mimic the impaired heart function resulting from a heart attack caused by a blockage of the coronary artery. The animals may be kept for several weeks to allow the changes to the heart to develop. They will probably be imaged to measure the changes to heart function and may be given a modified diet or drug treatment to determine whether this improves organ function and/or substrate metabolism. This treatment could include injection of stem cells into the heart muscle or attachment of a tissue-engineered scaffold across the damaged area of the heart to study the effect of adding new, healthy cells or tissue on the function and metabolism of the heart. Again, at the end of the experiment we will take tissue for *ex vivo* experiments as in protocol 1.

Protocol 5 is used to test which tissue-engineered scaffolds survive best after being implanted in the body, but without the complex surgery needed to access the heart. These animals will have scaffolds implanted under the skin in areas of the back where the scaffold does not affect movement. The animals may be imaged to measure the development of blood flow into the scaffold.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals will be used for method development on our imaging protocol. These animals will by anaesthatised several times, although normally this will be no more than once a week. However, the animals may become averse to the anesthesia over time.

Animals which undergo surgery to induce a heart attack may die during or after surgery. Once they have recovered, they rarely show signs of pain or abnormal behaviour or weight loss. We may keep these animals for up to six months as the heart gradually remodels after the surgery and we are interested to see whether therapies such as treatment with stem cells will mean that the heart function does not deteriorate so much over time.

We use animal models of diabetes which will have increased thirst and urination. The type 2 animals will become obese whereas the type 1 diabetic animals may show some weight loss. For most projects,

the animals will be diabetic for up to a month, but if we are looking at a potential therapy that takes longer to take effect we may keep the animals for longer. We use diabetic animals on our surgical protocol to induce a heart attack as we are investigating why diabetic patients recover less well. These animals may not recover from the surgery as well as wild-type animals and will be monitored particularly carefully.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We do a lot of experiments using the *ex vivo* perfused heart or isolated mitochondria. These animals will be on protocol 1 and will generally only undergo terminal anaesthesia. Some will have an overnight fast so that levels of metabolic substrates are not affected by a recent meal. Some animals will be used for method development on our MRI protocol and undergo general anaesthesia with administration of substances but no other interventions. These animals will predominantly have a mild experience although some on the imaging protocol may become averse to the anaesthesia and have a moderate experience.

Our type 2 diabetic model does not routinely result in more than increased weight gain and increased thirst and urination. We ensure that these animals have sufficient water and are on absorbent bedding and so many of these animals will have a mild experience. In some cases, higher levels of blood glucose will mean that the animals have a moderate experience. The GAA models of type 2 diabetes may develop increased levels of blood glucose and have a moderate experience.

The type 1 diabetic model will mean that animals develop high glucose and low insulin and these animals, either STZ-induced or GAAs, will have a moderate experience.

Animals which undergo surgery for myocardial infarction will have a moderate or severe experience, depending on how quickly they recover from the surgery and whether they progress to heart failure. Mice recover less well than rats and are more prone to developing life-limiting heart failure. Diabetic animals on this protocol are more likely to develop severe adverse effects.

Based on this previous experience, and the number of animals on each of our protocols, overall we expect around 50% of animals to have a mild experience; around 40% of animals to have a moderate experience and 10% of animals to have a severe experience.

What will happen to animals at the end of this project?

Killed

A retrospective assessment of these predicted harms will be due by 03 March 2026

The PPL holder will be required to disclose:

What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We study the interplay between organ function and substrate metabolism, with a particular emphasis on the heart. To obtain relevant measurements of heart function it is necessary to use an animal model with a four chambered heart like the human heart. As metabolism and contraction of the heart are inextricably linked, it is essential to study metabolism in the intact beating heart. Impaired delivery of metabolic fuels, due to decreased heart function or an imbalance such as is seen with diabetes, will mean that the organs of the body cannot function properly. This may, in turn, cause a further change in the metabolic balance of the body which could feed back to damage heart or liver function. As a result, these effects need to be studied in animals over time.

Which non-animal alternatives did you consider for use in this project?

We use mouse HL1 cells, a cell line that behaves like mouse heart cells, to determine the mechanisms behind effects observed in our *in vivo* studies. We also use this cell line to explore new theories before taking experiments into animals. We have developed a protocol to make the cells insulin-resistant and have found that these provide a good *in vitro* model of the changes we see in the diabetic rat heart. For example, we used these cells to investigate molecular changes occurring in diabetic animals after a heart attack, which lead to one of our recent publications. As it is not easy to get human heart cells, we use human stem cell-derived cardiomyocytes to help us determine whether effects seen in rats or mice are likely to also occur in humans.

In order to minimise the number of rodents used for developing cardiac imaging sequences at an early stage of the process, we are using an "eMouse", developed by collaborators, that generates a 'heart beat' and signals to mimic the movement due to breathing. We can mimic biological tissues, by adding chemicals to the gel, which has enabled the creation of "blocks" of tissue with much closer MR properties to certain tissue types of interest. This improves our ability to robustly test hardware and software prior to using it in animals.

Why were they not suitable?

The insulin-resistant HL1 cells provide a useful model but they only enable us to understand what is happening in heart cells in a monolayer, isolated from other cell types and from the mechanical stresses encountered by cells in the heart. We can generate engineered heart tissue, which is a 3D construct where the cells contract against flexible posts, and this is a better model of the environment cells encounter in vivo. However the cells retain a very immature phenotype and do not behave like a true adult heart cell. We are working to mature the cells and to adapt the insulin resistant model we use in the HL1 cells. Nevertheless, these cell systems can only ever provide a part of the picture when one is studying the complex metabolic interplay between different organs in the body. In addition, the heart contains many other cell types in addition to the beating cardiomyocytes. For example, after a heart

attack blood flow to part of the heart muscle is cut off so that the cells do not get enough oxygen or nutrients to continue beating efficiently. We can mimic this in our cell models. However the dying cells send out signals to the body's immune system and this attracts immune cells to the damaged region. Some of these immune cells are beneficial and others are not, and it is much harder to mimic the effects of this immune reaction in a dish. Non-beating cells in the heart, called fibroblasts, start to change and reinforce the dying muscle, forming a stiff scar and changing the workload on the rest of the heart which again it is hard to reproduce in a model.

Similarly, although the "eMouse" is extremely valuable for testing new hardware and software for MR imaging, it cannot provide the imaging problems associated with blood flow and movement due to breathing and does not provide any cellular or functional information.

A retrospective assessment of replacement will be due by 03 March 2026

The PPL holder will be required to disclose:

What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have been working with rodent models of disease for many years and use the information we have from previous studies to determine the number of animals we think will be needed for particular studies. Based on our current and planned funding, we know which types of experiments are proposed for the next few years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We take statistical advice from experienced researchers in the field, combined with our own previous experience of working for over 15 years in this area. We use statistical power calculations to estimate the number of animals we need in each experiment to detect metabolic and/or functional changes. We have developed new statistical methods that are unique to MR experiments that others in the field now use, which increase statistical power. Where we are testing a new drug compound, after appropriate *ab initio* calculations, we will use a small number of animals to determine the dose of drug to give before undertaking the experiments with sufficient animals to determine whether the drug has the effect we predict. In all cases we aim to pick the animal model which provides consistent results in our experiments, to reduce the number of animals we need.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use MRI to measure cardiac function and metabolism on the same animal over time as the disease progresses. The MRI does not affect the animals adversely and allows us to reduce the number of animals required to follow the disease progression. We can measure substrate metabolism *in vivo* in the heart and the liver in the same animal, further reducing the number of animals needed; and we have refined our *ex vivo* heart perfusion measurements so that we can measure metabolism of both glucose and fat in one heart where previously we would have needed to use of two animals.

Where possible we use cryoinjury in the rat to provide a damaged region of heart tissue. The previous ischaemia/reperfusion protocol meant the animal was kept under anaesthesia about 90 minutes and also resulted in a large variation in tissue damage. The new protocol comprises placing a liquid nitrogen-cooled probe on the heart tissue for 10-15 seconds and means that the animal is under anaesthesia for about 30 minutes. This protocol produces a more reproducible infarct size and therefore results in fewer animals being used. It is also a refinement because the animals are under anaesthesia for about 30 minutes rather than 90 minutes.
However, the type of damage does differ from that resulting from a blocked artery and so in some cases we have to use the previous protocol where we block the blood flow through the artery, either permanently or for just under an hour.

We have been using omics-based technology to investigate the metabolic changes occurring in diabetes or after chemotherapy. We have discovered that the high rate of reproducibility and low error measurements from these mass spectrometry-based techniques have allowed us to decrease our numbers needed to find the information we need.

Where possible, we use tissue or organs from control animals not required for the designated study, or animals that do not fit the criteria for the designated study, for method development on other studies, such as imaging protocols or *ex vivo* mitochondrial analysis.

A retrospective assessment of reduction will be due by 03 March 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use rats and mice because the hearts and other organs are structurally and metabolically close to the human heart. Although we have to generate disease models for our experiments, we use non-invasive techniques wherever possible, for example we feed high fat diet to elevate plasma free fatty acids and give therapeutic compounds in drinking water if applicable. We use a model of cryoinjury to induce damage to the rat heart but maintaining an open artery, as is seen clinically, because this generates more reproducible results, thereby reducing the number of animals needed, and exposing animals to a shorter period of anaesthesia than required for ischaemia/reperfusion. For experiments to design cell scaffolds for heart repair, we first test whether these are tolerated by the body, by implanting them in a pocket under the skin, before we progress to the more invasive surgery to attach scaffolds to the heart.

We use genetically altered animals (GAAs) when we are investigating the role of a specific gene in a disease pathway or to mimic a particular aspect of a disease. In general, mutations in metabolic pathways may cause the animal to gain or lose weight but do not cause significant pain or suffering. If the disease phenotype may deteriorate with age we will use the animals before they reach that point.

Why can’t you use animals that are less sentient?

In order to examine changes to metabolism and organ function in disease, we need to use a model that matches the human physiology, rather than a non-mammalian system. For some of our experiments we take a wild type animal and terminally anaesthetise it before imposing metabolic changes on an isolated organ. For these experiments, we need to perfuse the heart in a system which mimics the delivery of oxygen and substrates and for this we need a heart from an animal the size of a mouse or rat. Similarly for our MR experiments we need enough tissue to get a measurable signal from the heart. These experiments would not be possible in a species that is the size of a fish.

For most of our *in vivo* experiments, we look at how a disease such as diabetes or a heart attack changes the function of the heart over a period of weeks and we cannot keep an animal terminally anaesthetised for that length of time.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have purchased a Mouse Monitor from Indus Instruments that includes a heating pad and records ECG, body temperature and pulse oxygenation from both rats and mice. We use this to monitor animals during surgery and it has been very useful whilst establishing the cryoinjury model, as we can detect changes on the ECG which can indicate whether that the damage to the heart is too severe for the animal to be safely recovered.

We've introduced double-HEPA filtered air handling systems as much as possible into our workflows, both MRI and otherwise. As well as protecting operators, these will have positive beneficial effects on rodents undergoing procedures: as well as increasing sterility, there is a lower likelihood of airborne scents being communicated between animals.

A dedicated rodent echocardiography machine has been purchased for cardiovascular research, which is

more efficient for scanning animals than human systems previously used, and therefore can achieve better results with less time under anaesthesia for each animal.
This will be particularly useful for screening animals after myocardial infarction or aortic banding where the surgery can give variable results. Where the surgery is unsuccessful or the effect is too severe, the animal can be removed from the study at an early time point. Similarly, we monitor blood glucose levels regularly, using a pinprick sample of blood and a glucose monitor. Again, where an animal is not sufficiently diabetic or blood glucose levels are too high, the animal can be removed from the study at an early time point.

In order to observe breathing motion of the animals in the MRI scanner, we have previously used an induction loop across the chest. This loop is effective, but the signal it provides can be obscured briefly during each MR pulse. We have recently installed a small warm plastic balloon, to be placed underneath the animal, that enables more robust monitoring of breathing, thereby increasing scanning efficiency and decreasing the time the animal is under anaesthesia.

We have close links with the cardiac groups at the Wellcome and other imaging groups in the University and discuss techniques and share hardware and software advances between the groups. We liaise with the veterinary staff and keep up-to-date on LASA guidance to minimise harms.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will use the NC3Rs experimental design assistant when planning our experiments and will follow the LASA guidelines on aseptic surgery. We will keep up-to-date on advances publicised in the NC3R newsletter which provides information on the most refined techniques, such as new guidelines on non-aversive methods of picking up animals, single-use of needles and blood sampling. We will adhere to updated ARRIVE guidelines on reporting work with animals as now required by many journals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The PPL holder or a designated representative attends the departmental 'gold standard meeting' each term at which the 3Rs are discussed. Members of the team attend workshops and courses run by the NC3Rs and all staff receive the NC3Rs newsletter from the Home Office Liaison team. Relevant advances from these outlets and from reading the literature are discussed at the weekly lab meeting.

A retrospective assessment of refinement will be due by 03 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

7. Assessing novel genetic therapies in neurological disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

gene therapy, neurodegeneration, genetic brain disorders, molecular therapy, genetic rodent models

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Rats	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To develop and test new genetic therapies for currently untreatable brain disorders.

A retrospective assessment of these aims will be due by 26 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Faulty genes are a significant cause of severe brain disease, especially those affecting children. We are currently undergoing a revolution in how we understand and envisage treating highly debilitating genetic disorders of the brain. The focus of this work is on childhood onset neurological disease for which there are no current effective treatments. Genetic brain diseases are often lifelong, affect a considerable number of individuals globally and place a huge socioeconomic burden on families, carers and health services. Childhood onset brain disorders are especially costly with regards to treatment, lost earnings, care and support for children and adults. The revolution in genetic medicine now means that we are diagnosing these diseases and their underlying genetic causes very effectively. However, this is not matched by treatment options and it remains the case that there is no effective therapy for the majority of severe brain disorders. That said, advances in gene therapy (replacing or fixing a faulty gene) offers the option of developing genuinely transformative treatment options. The reason that genetic approaches to therapy has such potential is that they offer to fix the root cause of the disease rather than just some of the symptoms. The goal of this programme of research is to develop and establish the effectiveness and safety of gene therapy across a number of significant brain disorders. For this we use rodents in which the equivalent gene that causes brain disease in humans had been disrupted or inactivated. Assessing the impact of gene therapy drugs in such animals allows us to predict the extent to which the gene therapy is likely to be safe and effective in people with severe brain disorders.

What outputs do you think you will see at the end of this project?

This project will provide vital new data on the potential utility of gene therapy in pediatric onset neurological indications. Based on our work to date, it can be expected that outputs include publications, public dissemination, patent applications and lead molecules for preclinical and clinical development.

Who or what will benefit from these outputs, and how?

There is currently intense research in the genetic therapy space and any early publications on innovative therapeutic approaches or tractability of disease phenotypes will be of significant impact in the short to medium terms (1-3 years). Data generated by this project will allow us to refine our approaches to hone in on the most likely effective interventions in childhood onset severe brain disorders. The longer terms benefit of these outputs, especially patents and therapeutic molecules will be new treatment options in hitherto untreatable pediatric disorders. We have a track record in this area with genetic therapies we have developed being destined for first in human gene therapy trials. Beneficiaries include patients of childhood genetic brain diseases and well as scientific and medical researchers in this fast moving field.

It is expected that the innovative genetic therapies proposed will facilitate the rapid advances being in this sphere of translational research and will have direct potential for very rapid (in drug development terms) therapeutic impact. In addition, the parasite therapy is a highly innovative therapeutic approach which is much earlier in terms of translational development but has the potential to deliver therapeutic cargoes that are difficult to deliver by established methods. It thus has the potential to treat patients with mutations in very large or complex genes or even multiple genes.

How will you look to maximise the outputs of this work?

It is vital to the ethos of the laboratory that we work in collaboration with other laboratories both within and without the research institution. We have established links with laboratories across the UK and internationally with whom we share new knowledge, techniques and ideas. This informal 'peer review' process enables us to interrogate paradigm and experimental designs, enabling us to target those experiments we feel will generate the most useful and transferable.

Species and numbers of animals expected to be used

Mice: 10000
Rats: 1800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will require the use of animal models that exhibit the clinical features of patients with neurological disorders under investigation. As our focus is on assessing potential treatments for pediatric and juvenile-onset disorders, we require the use of rodents at neonates, juvenile and adult stages of development. Rodents used in this work are genetically altered to model genetic brain disorders, with rats and mice being well suited for assessing cognitive (learning and memory) and functional outcomes.

Typically, what will be done to an animal used in your project?

Experimental animals will be enrolled in therapeutic studies. Animals will be assigned to a treatment group and novel genetic therapies administered, typically via a single injection. Animals will then be monitored for clinical signs of neurological disease to assess whether the therapeutic intervention impact aspects of the disease. Tests may include assessment of activity, walking, learning and memory, anxiety or functions such as breathing control.

What are the expected impacts and/or adverse effects for the animals during your project?

The therapeutic agents being tested are known as gene therapy and target the root-cause of the diseases under investigation. As such, there is an expectation that the therapy may be highly impactful in ameliorating the clinical features of the disease. We expect that the therapy may result in correcting highly debilitating features of neurological disease including motor function (control of muscles), breathing control, seizures as well as higher level brain functions such as cognition. It is possible that we will observe unexpected adverse effects due to the novel therapeutic agents. Where these occur, experiments will be stopped immediately.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For the majority of the work the severity will be in the mild to moderate range, especially in neurological

disease models where the main clinical feature is intellectual disability. In some instances, the rodents will model very profound neurological disease and in order to assess the impact of gene therapy in these instances, the procedure will be considered severe.

What will happen to animals at the end of this project?

Killed

A retrospective assessment of these predicted harms will be due by 26 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The neurodegenerative diseases under investigation in this project are complex conditions exhibiting a range of physical, neurological and learning impairments. We aim to test the impact of gene therapy on these complex aspects of neurological disease. As such it is not possible to model these conditions in isolated systems such as single cells grown in a dish. Therefore, the use of animal models is unavoidable when investigating these disease types.

Which non-animal alternatives did you consider for use in this project?

We have made significant efforts to replace experimentation on live animals to investigate complex neurological disorders. This includes the use of neuronal and other stable cells lines for the testing of genetic therapies at a cellular and molecular level. We also study gene structure and sequences using human post-mortem tissues. We collaborate with groups working on human-derived cell lines from patients with intellectual disability disorders.

Why were they not suitable?

None of these alternative approaches enable the assessment of gene therapy on key neurological features that characterize childhood-onset neurological disorders such as intellectual disability or motor control. Instead, the therapeutic potential of agents in neurodevelopmental and childhood-onset disorders require the study of an intact nervous system as they involve combined problems in brain circuitry, connectivity, neurochemistry and brain cell physiology which develop over time. It is only by creating accurate genetic models of these disease which closely mimic the major features of the human condition, that one can study how these processes interact. Moreover, whilst cellular level assays are used to test whether pharmacological and genetic interventions can rectify disease at the single cell level, it is only by testing these at the whole brain/animal level that one can truly assess whether these translate to improvements in behaviour, learning or corrected brain development.

A retrospective assessment of replacement will be due by 26 May 2026

The PPL holder will be required to disclose:

> What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from yourexperience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The principles of our experiments are twofold. One is to characterise the development of the disease in the nervous that underlies Rett syndrome and related disorders so that we can identify avenues of potential therapy. This mainly includes the use of genetically altered animals and wild-type controls to provide tissues necessary for investigation outside the animal, such as neurochemical, molecular, electrophysiological and anatomical profiling. The second is to utilise this knowledge to develop and test the efficacy and safety of therapeutic interventions. Whilst as much of this as possible is done at the cellular level, for instance testing genetic therapies in cultured cells, Rett syndrome is fundamentally a nervous system disorder and for this we need to test putative therapies in the whole animal.

Our research conducted using animal tissues (electrophysiological, biochemical and morphological assays and genetic and pharmacological interventions) is based on the minimum number of animals required to produce sufficient biological replicates for robust statistical comparisons. Our use of cultured cell systems enables the preparation of multiple cell-based assay systems from a smaller number of animals.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have developed cellular assays to characterise the properties of our molecular therapies prior to any animal work. This acts as a triage even prior to any pilot rodent study. Where organismal level work is required, we will use the minimal numbers to achieve robust scientific outcomes. All experiments are conducted according to the ARRIVE guidelines.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For experiments on living animals, our previous experience has shown that 15 animals in each control and experimental group will provide a 90% chance of detecting a significant change in respiratory, motor and other behavioural phenotypes. We employ the services of a professional biostatistician to advise on all areas of our gene therapy work.

Pilot experiments on a small sample of animals (maximum n=5) will be conducted to select appropriate drug doses and for viral studies, to assess effectiveness of transgene expression.

A retrospective assessment of reduction will be due by 26 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The majority of experimental procedures will be performed on mouse models of RTT. In additions, mouse models of CDKL5, Syngap, Pten deficiency, Angleman syndrome and other NDD models will be used during this project. We intend to develop gene therapy treatments for RTT which will inform our approach in developing gene therapies for other conditions. Whilst RTT-like phenotype is severe in rodents, our experienced and knowledgeable scientists will closely monitor the animals and will take the following steps to ensure that pain, suffering and distress are kept to a minimum.

We have adopted a revised phenotype scoring system which we use at least weekly to assess, in a semi-quantitative and objective fashion, the onset and development of RTT-like symptoms in mice and rats. We have refined the scoring system so that there are now two separate instruments - while sharing most of their criteria, one is deployed to assess the phenotype of the mice for use in analysis, and the other is deployed to assess whether welfare-related actions are required, including closer monitoring, a vet-supported husbandry package, and clearly-defined humane endpoints.

Male Mecp2 mutant mice have an early-onset phenotype that becomes progressively more severe. They are therefore not kept alive long enough to become severely symptomatic unless required for a specific experiment. Treated mutant mice used to provide tissue for cell culture and other ex vivo experiments are euthanised while still presymptomatic.

Neonatal dosing is used to assess early genetic interventions in NDDs. Our breeding strategies generate mixed litters of GA and non-GA animals. We have therefore adopted a rapid genotyping protocol to identify mice with the appropriate genotype and avoiding unnecessary injection of the wildtype mice. For this approach it is necessary to conduct tail biopsies.

Gene therapy is generally considered an irreversible treatment in that, once delivered, the treatment cannot be withdrawn. Part of our programme of works is to develop safety features whereby a pharmacological agent can be used to turn down/shut-off the transgene expression at the earliest sign of any adverse effects. This is a safety feature for translational purposes but is also a refinement during preclinical testing of gene therapy cassettes to mitigate against potential adverse effects in mice.

Use of rat models: In addition to mouse models, rat models of CDKL5 deficiency, SynGAP deficiency and Fragile X syndrome will be assessed. Models of these disorders show less overt outward phenotypes (motor, autonomic and peripheral phenotypes) and are therefore more dependent on detecting more subtle behavioural phenotypes that are more clearly expressed in rat models. In terms of refinement, this enables the finer grain testing of therapeutic interventions in domains (e.g. cognitive) that are most pertinent to the disorders in patient. It is a key scientific objective to assess whether cognitive features are reversed by genetic therapies targeting Rett syndrome and for this reason we wish to extend experiments into rat models which enable the assessment of more robust behavioural outcomes. For SynGAP knockout rodents, the phenotype is severe in hemizygous male animals. In limited proof-of-concept experiments, we wish to establish whether early genetic intervention can ameliorate the onset of these overt phenotypes in male animals. As a refinement, we would aim however to progress the study to heterozygous females as quickly as possible. All rat studies will be in collaboration with groups within the centre that have existing extensive expertise in dosing, maintaining and phenotyping rat lines.

We aim to also test an unconventional approach to deliver therapeutic protein to the brain by delivering engineering attenuated strains of Toxoplasma that can secrete the MeCP2 protein into neurons. Having shown that this approach is effective in delivering MeCP2 to neurons in vitro, we reply on experiments in mice to show that the approach has therapeutic application in vivo in terms of establishing whether this delivery approach can impacton RTT-like phenotypes.

Why can’t you use animals that are less sentient?

The use of genetically-modified mice is frequently the only viable strategy for studying neurodevelopmental genetic disorders. Indeed, they are especially well suited for studying single-gene disorders of brain in which accurate genetic mouse models recapitulate many of the cardinal features that characterise the disorder in humans. Intellectual disability is characterised by impaired cognition and at present this can only be tested in the live animal. However, we have developed sensitive assays for changes in motor function that can detect nervous system abnormality at a very early stage (at 4-8 weeks). This enables us to follow the trajectory of the disorder phenotype from an early stage and assess whether therapeutic interventions affect the onset and development of disease-like characteristics rather than simply testing for the reversal of established and more severe symptoms.

RTT is a severe neurological disorder and is diagnosed once patients develop overt symptoms. The aim of the research is to develop effective therapies for RTT. As such, animals are therefore required to develop overt RTT-like signs in order to test the impact of putative therapies. Protocols 5, 8 and 8 are categorised as severe for this reason.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

As detailed in the project plan, we have implemented a very rigorous monitoring system for our Mecp2 rodent lines. This include regular detailed monitoring of welfare and an escalation of monitoring frequency and enhanced husbandry (bedding, soft food etc) as the phenotypes progress as well as

clearly defined humane endpoints. Any of studies involving surgical or invasive intervention will adopt appropriate pain management and post-operative care. For mouse lines that have a propensity for benign tumours, only young mice will be maintained unless on a dosing procedure. In these cases, mice will be monitored frequently and with clear humane endpoints upon tumour discovery.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidance on the Operations of ASPA - https://www.nc3rs.org.uk/

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our technical staff are members of professional bodies (Institute of Animal Technology, Royal Society of Biology) and take part in continuing professional development to ensure their knowledge is as current as possible. Regular updates from bodies such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and Understanding Animal Research (UAR) mean new developments and best practices can be evaluated and implemented where they might improve animal welfare without inhibiting the integrity of the research. Regular communication with our veterinary staff will ensure techniques are reviewed and refined where necessary and appropriate.

A retrospective assessment of refinement will be due by 26 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

8. Assessing Novel Treatments for Endometriosis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Endometriosis, Preclinical model, Fertility, Drug therapy, Inflammation

Animal types	Life stages
Mice	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our aims are to gain a better understanding of endometriosis and to assess the efficacy of new treatments to prevent disease growth and recurrence.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Endometriosis is a debilitating condition that affects an estimated 176 million women worldwide. In endometriosis, cells similar to the lining of the womb grow elsewhere in the body, typically in the abdomen around the ovaries and the bowel. These endometrial-like cells respond to female sex steroids with a chronic inflammatory reaction that is the leading cause of pelvic pain and infertility. Often dismissed as women's troubles, a lack of research and funding means sufferers can live in severe pain, unable to work or socialise. Current drug therapies carry side effects, are not suitable for patients seeking pregnancy and endometriosis often recurs upon discontinuation of drugs. There is a scientific and clinical need for a better understanding of endometriosis development/recurrence and the assessment of new treatments/delivery systems.

What outputs do you think you will see at the end of this project?

New information

It will increase our understanding of the disease. In particular, it will provide new information in relation to the local peritoneal environment (the abdominal area surrounding the lesions) in disease progression and following treatment with novel compounds. In this programme of work, we will also look to develop and investigate a model of recurrence and utilise this model to assess treatments.

Specifically, lesion size, hormone production, inflammation and metabolic pathways known to exacerbate the disease will be studied. This work should increase knowledge about the effect of new treatments and modalities on endometriosis development, progression and recurrence. It is anticipated that these treatments and delivery methods will reduce lesion formation and/or prevent their regrowth, thus providing important information with the aim to progress these compounds to the clinic for patients.

Information in relation to novel drug delivery platforms, including the suitability of hydrogels, will also be assessed. These modalities have yet to be studied as a local delivery system within the peritoneal cavity, where lesions are usually found. Surgery will be performed at similar times of the day to avoid body clock effects on inflammation, adhesion and local repair.

By monitoring the reproductive cycle via vaginal lavage and cytology, drug effects on reproductive function will also be determined. This measures the integrity of hormone signalling, that may indicate local verses systemic drug release. It will also show if contraceptive effects are reversible when treatments are discontinued.

Publications

Our research spans across many different scientific fields, including: endocrinology, pharmacology, pharmaceutics and material science. We plan to submit articles to high impact, open access journals, such as the British Journal of Pharmacology and the Journal of Controlled Release.

There is limited previous research on endometriotic lesion recurrence; therefore, this research is very novel. Publications will demonstrate validity of our model of recurrence as well as the effect of novel and/or localised treatments.

There is also a sparsity of hydrogel imaging research within the peritoneal/abdominal cavity, which would be of interest to drug delivery scientists studying diseases within that area of the body.

Products

If proof of concept is successful and lesion weight or endometriosis recurrence is significantly reduced, novel treatments and/or hydrogels could be considered as a possible, and viable, treatment option within the clinic.

Who or what will benefit from these outputs, and how?

Scientific community

Studying the local lesion environment with or without drug treatments would enhance knowledge about the cause of endometriosis and its development, progression and recurrence. This information would benefit other researchers within the same field as well as the general scientific community and general public.

Translation for clinical benefits

Successful placement and efficacy of the hydrogel-based delivery system could have clinical benefit for women with endometriosis. Introducing a localised drug delivery system as opposed to oral or injection-based treatments could reduce the severe side effects that often occur with systemic dosing, such as: weight gain, headaches, depression, reduced bone density and acne. Current intra-uterine devices carry risks of ejection from the body, infection and piercing of the uterus that would be mitigated by the hydrogel. By localising drug release, women receiving this treatment could have the option to conceive, which is not feasible with current hormonal therapies. Targeted delivery would therefore improve patient compliance, reproductive health and would also reduce the need for multiple surgeries. This would significantly cut healthcare costs, which are currently estimated at £8.2billion/annum in the UK alone, by saving the need for referrals, specialist centres, additional treatments and lost working time. The clinical benefit of these new medicines would be more long-term, beyond the lifespan of this project licence.

How will you look to maximise the outputs of this work?

We are collaborating with other research groups concerned with the peritoneal/abdominal cavity, regenerative medicine, imaging experts, gynaecologists and material engineers. This collaboration will allow for hydrogel formulation, live imaging and analysis of suitability for the clinic. This will provide

information on both drug release to endometriotic lesions as well as the prevention of adhesions, which commonly occur within this area of the body, particularly after surgery.

To avoid publication bias, we are committed to publishing all findings from this project, both positive and negative. Outputs will be maximised through publication of abstracts, papers, conference presentations, patient public involvement and public engagement activities.

Species and numbers of animals expected to be used

- Mice: 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We require our chosen model to undergo an oestrous cycle, the equivalent of a human menstrual cycle. Hormonal changes, particularly in oestrogen, are essential for disease establishment and progression. We also wish to monitor if our chosen localised treatments disrupt this oestrous cycle. Therefore, it is necessary that the chosen animal is a sexually mature mammal. As a group, we have chosen to utilise mice.

Typically, what will be done to an animal used in your project?

There are two main categories of animals used within this project: donor mice and recipient mice.

Donor Mice

Donor mice will be humanely killed in order for their uterus to be removed and sectioned into small pieces. In order to analyse which phase of the oestrous cycle these mice are in, a vaginal wash will be conducted on these mice prior to being humanely killed.

Recipient mice: disease establishment

In all cases, recipient mice will undergo surgery under anaesthesia. The uterine fragments from the donor mice will be stitched onto the peritoneum of the recipient mice, where lesions commonly form in humans. Recipient mice will undergo a small incision of the skin and muscle layer in order to gain access to the cavity. Prior to surgery, these mice will be injected subcutaneously with opioid-based pain relief. The wound will be cleaned with betadine solution and their eyes will be treated with lacri-lube to prevent dryness/irritation. Recipient mice will then be split into key groups: systemic vehicle, systemic treatment, localised vehicle and localised treatment. Vehicle will be the same solution the chosen drug is suspended in. Mice that are treated systemically with treatment or vehicle will undergo repeated administration throughout the project. Depending on the drug type, this will be given either by injection or orally. Recipient mice given localised vehicle or treatment will not need these repeated doses as a

hydrogel will be administered during surgery. Some mice will have small volumes of blood taken to analyse drug exposure levels. A proportion of these animals will be humanely killed at specific time points to allow for extensive analysis of the lesions as well as the surrounding area.

Recipient mice: established lesions and disease recurrence

In order to analyse drug effects on established lesions or lesion recurrence, these recipient mice will undergo two surgeries. They will undergo the surgery as detailed above to establish endometriosis within the animal. These animals will then be allowed to recover for a period of 2 weeks. After this time, the animal will undergo a second surgery which is almost identical to the first. Some of these animals will have their established lesion removed, whilst others will not. Treatment will start at this stage, with the same groups still applying: systemic vehicle, systemic treatment, localised vehicle and localised treatment. Animals receiving localised vehicle or treatment will be given the hydrogel at the time of the second surgery. Animal receiving systemic treatment or vehicle will be given either repeated injections or oral doses. Some mice will have small volumes of blood taken throughout the experiment to analyse drug exposure levels. Vaginal wash will also be performed to determine drug effects on reproductive function. A proportion of these animals will be humanely killed at specific time points to allow for extensive analysis of lesion regression or recurrence.

Live imaging

Recipient mice from each of the two procedures described above may also undergo non-invasive, live fluorescent imaging. This will allow hydrogel localisation, lesion growth and recurrence to be monitored.

What are the expected impacts and/or adverse effects for the animals during your project?

The expected impacts of both protocols is the development of endometriotic lesions, which have been shown to establish within roughly 2 weeks after induction. As the model of endometriosis proposed within this project has been used and verified in the previous project licence, it has been shown to be well-tolerated by the animals, with no outwards sign of pain or distress.

Drugs previously tested within this animal model appear to also be well-tolerated by the animals with no visible adverse effects. Drugs that have yet to be tested within this model are well characterised within the literature.

Localised drug delivery vehicles being used within this project, namely hydrogels, are also well-characterised within the literature and appear to not have a toxic effect in numerous cell types/lines. This will be verified with our cells of interest within the laboratory prior to commencement of any animal work.

Daily vaginal wash was well-tolerated by the mice when performed for up to 4 weeks under our previous licence.

A potential impact of protocol 2 is aberrant wound healing due to second surgery. However, this will be assessed in a small pilot study to see potential adverse effects and their duration.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Donor animals used (~20%) are humanely killed using Schedule 1 (mild).

Recipient mice (~80%), the maximum expected severity is moderate.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Endometriosis is a complex disease with a relatively poorly understood cause. Past research has shown that the cause of endometriosis has a hormonal, immune and inflammatory element. Therefore, in order to correctly analyse the effects of localised treatment, an animal model is the only viable option as it takes into consideration all of these options. Work using cells or individual organs fail to account for all of these components.

Which non-animal alternatives did you consider for use in this project?

Prior to animal work, primary cells as well as cell lines have been utilised to monitor the effects of these drugs as a solution and within a hydrogel. Cells of interest and surrounding cell types were studied. Cell-based experiments on how to investigate cellular regrowth are also being investigated.

Studies investigating drug release from the hydrogels are being conducted within the laboratory, using media similar to the peritoneal/abdominal fluid.

Why were they not suitable?

Whilst methods involving primary cells and cell lines gave information on drug efficacy in cellular models, it does not provide detailed information on these drugs effects on interacting cell types and the complex environment surrounding the lesions. Laboratory methods used to monitor drug release only takes into account the diffusion of the drug. It fails to account for degradation of the hydrogel by enzymes and shear stress.

For this, we need a more complex, animal-based system to be able to monitor how these drugs are effective and can prevent disease progression/recurrence.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Working alongside a trained statistician, we utilised data acquired from previous animal work conducted within this group to calculate the minimum number of mice needed per treatment group and lesion number per mouse to find a biologically relevant reduction in lesion weight. Lesion weight was used as it is the most useful and clinically relevant measurement in the studies. This analysis allowed us to account for variation than can occur both between and within mice. It allowed a study with best chance of detecting meaningful outcomes to be designed.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have utilised the NC3Rs experimental design tool in order to design our animal-based experiments. This allowed us choose appropriate groupings and blind the studies effectively. This design tool has also helped us identify any potential nuisance variables and how to avoid them. By doing this, we are reducing the likelihood of experimental replication, thereby reducing the number of animals used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Donor uterus is segmented into many fragments (typically 12-15 pieces), therefore, fewer donor mice are required than recipient mice. Typically 3 lesions can be induced within a single recipient mouse. This reduces the numbers of recipient mice needed to see an effect of the drugs on lesions.

We have conducted pilot collaboration work to determine whether fluorescently-tagged hydrogels can be monitored within the peritoneum using live imaging. This pilot study investigated two different types of fluorescent tags. We humanely killed a small cohort of animals, from there, we conducted the study as planned in the protocol. This study confirmed that the hydrogel can be imaged within the mice, which means there is a reduced need for sacrifice at specific time points.

We will also conduct a small pilot study, which will involve analysing wound healing from dual surgery. This will provide information on the rate of wound healing and will ensure that dual surgery is feasible.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare

costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will be using a mouse model to mimic endometriosis. This model requires both donor and recipient mice. Donor mice are humanely killed to allow for their uterus to be removed and segmented into small pieces. These segments are then stitched on to the peritoneum of recipient mice. This particular model will be used as it is one of the most well-established models of endometriosis within the literature. Previous work in our laboratory found that this method produced fluid-filled cysts that are characteristic of the disease. The cysts were also found to be responsive to hormones, which is also one of the main disease characteristics.

For the recipient mice, this technique requires a small incision on the ventral area, limiting pain and potential harm to the animal. From previous work, the animals recover well with no obvious adverse effects in relation to pain or distress and in the vast majority of animals we did not observe any changes in weight or normal behaviours.

Our previous work demonstrated that following surgery the lesions develop naturally without supplementing animals with hormonal treatment. We also conducted ovariectomies (removal of ovary function) in our original work to prove hormonal dependence of lesion development. These previous studies have allowed us to further refine our methodology and both steps have been removed from our studies.

Why can’t you use animals that are less sentient?

We require the mice for this project to be sexually mature mammals, as changes within the oestrous cycle are important to monitor and can affect lesion establishment, growth and recurrence. Therefore, using immature life stages or less sentient animals, i.e. non-mammals, is not scientifically appropriate.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be allowed to acclimatise to their environment to reduce any distress. Animals will also be trained to become accustomed to regular and appropriate handling.

For each step appropriate controls are in place to minimise any adverse effects for the animals and humane endpoints are clearly defined. Animals will be monitored by both the research group and the unit staff.

Animals will receive appropriate pain relief pre- and post-operatively. However, non-steroidal anti-inflammatory drugs will be avoided as the inflammatory response is involved in disease development.

Following surgery, animals will be allowed to recover from anaesthesia in a temperature controlled incubator. Surgical wounds on the animal will be subjected to after-care such as betadine solution to prevent infection. Lacri-lube will also be administered to each animal to prevent eye dryness and irritation whilst anaesthetised. Following recovery, animals will be closely monitored, weighed regularly and observed for any signs of distress or pain. We have also refined our animal handling techniques in line with current guidance.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the best practice guidelines highlighted within the document 'Responsibility in the use of animals in bioscience research' which is published by the NC3Rs.

We will also be following the best practice guidelines published by the NC3R concerning: blood sampling; grimace scale; how to pick up a mouse; housing and husbandry; procedures with care and rodent welfare hub.

We will comply with local practices and keep up to date with newsletters and bulletins sent by the animal unit.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly consult with our named information officer in order to stay knowledgeable in advances of the 3Rs. As well as this, we will keep up to date with any relevant literature on the 3Rs or published papers from the NC3R to ensure our work will always be within the recommended guidelines. We will also keep up to date with any news and bulletins provided by the animal unit in which we are undergoing our research.

Home Office

NON-TECHNICAL SUMMARY

9. Autoimmune and inflammatory diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Autoimmune, Inflammation, Arthritis, Inflammatory bowel diseases, Therapy

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To identify and investigate novel molecular targets and mechanisms and develop new medicines for the treatment of autoimmune and inflammatory diseases.

A retrospective assessment of these aims will be due by 08 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Despite the progress in the discovery and development of medicines, there remains a significant unmet need in the treatment of autoimmune and inflammatory diseases. Such diseases contribute to more than 100 serious chronic illnesses involving almost every organ system in the human body, affecting an estimated 7-9% of the world population, and this incidence has been rising by 3-9% over the last few decades. Women account for 80% of those individuals with autoimmune diseases (Invernizzi et al., 2009). For example, women account for 80–95% of patients with primary Sjögren's syndrome, systemic lupus erythematosus (SLE), and about 60% of arthritis patients are women (Beeson, 1994). Autoimmune diseases present a group of common and highly disabling long-lasting conditions with similar features making diagnosis and treatment extremely difficult, which are among the world's leading causes of death. Autoimmune and inflammatory diseases are frequently associated with additional serious healthcare issues, including severe pain, fatigue, depression, anxiety, chronic morbidity presenting a considerable burden on healthcare systems. In addition, a patient with an autoimmune disease such as rheumatoid arthritis or IBD, is significantly more likely to develop others, leading to more complex healthcare needs and lives that are considerably more challenging.

Autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), Sjögren's syndrome (SS) and psoriasis are all characterised by a breakdown in function of the immune system and/or activation of pathological mechanisms that remain poorly understood. These diseases vary greatly in the tissues and/or organs they affect and how they are presented, with some being limited to a particular tissue and others affecting the entire body. Through the assessment of information obtained from individuals living with autoimmune and inflammatory diseases, identification of the biological mechanisms and specific genes involved can be identified as novel approaches for treating such diseases. The use of animal studies is

fundamental for the validation of these mechanisms and genetic targets to support the research and development of potential new medicines.

In addition, the production of autoantibodies in autoimmune diseases has a profound involvement in the development of pain associated with such diseases. Cellular responses involved during acute inflammation leading to activation of the immune system, trigger mechanisms involved in pain signalling. However, recent studies suggest that the pain associated with autoimmune diseases such as RA develops in the absence of inflammation and is primarilly driven theough the production of autoantibodies (Wilderbrand et al., 2016). Although there is a large variety of autoimmune disorders with different symptomologies, pain appears to be a common factor in most of these conditions. The pain associated with autoimmune diseases is considered as one of the leading causes of comorbidities such as depression, resulting in a significant impact on quality of life and disease burden.

Increased understanding of the immune system and the mechanisms involved in the development and maintenance of autoimmune and inflammatory diseases has resulted in the important discovery of many effective treatments that manage the symptoms, but have little or no effect on the underlying causes of the disease. In addition, with undesirable adverse effects and a high proportion of non-responders associated with current treatments, there still remains a high unmet need to develop new medicines that are effective against multiple autoimmune and inflammatory diseases, offering a significant improvement over currently available treatments.

What outputs do you think you will see at the end of this project?

This licence supports a wide range of methods to enable the identification and evaluation of inflammatory and immunological systems involved in autoimmune diseases, to enable the investigation and development of potential new medicines to act against those diseases. The information generated from this work will enable scientific programmes to increase understanding of how mechanisms could drive development and maintenance of disease or why a condition may not heal or resolve. Studies targeting the identified and validated biological mechanisms will enable the effectiveness of therapeutic agents to be tested and developed as new medicines. This will provide information on common mechanisms that underlie different systems and disease indications (to enable subsequent expansion of research into a wider range of disease indications when mechanisms in common exist). It is also anticipated that results generated from these studies will be shared through scientific meetings and literature to further increase scientific knowledge and understanding in this field.

Who or what will benefit from these outputs, and how?

The information generated from this work will contribute to the development of new medicines to address the unmet medical need and improve the lives of people suffering with autoimmune and inflammatory disease.

Early research and discovery efforts will continue with the identification and validation of new disease related targets, which will form the basis of continued discovery and development of novel and effective therapies to benefit patients and increase knowledge available to the scientific community. Patient

benefit will occur over the longer term due to the long timescales involved to develop new medicines. The wider scientific community will benefit from published work presented at scientific meetings and in scientific journals to further increase knowledge and understanding in the field.

How will you look to maximise the outputs of this work?

It is expected that the information generated from the work carried out under this licence will be included in scientific publications as part of the process of investigating and validating new disease mechanisms and targets, and developing new treatments for disease. Where work is considered to be 'pre-competitive' (e.g. method development or model validation work), whereby it does not contain information that is subject to intellectual property constraints, it will also be considered for publication. In addition, the company also supports the view that publication of unsuccessful approaches ('negative data') is a valuable scientific output from properly conducted research, and this type of data would not be excluded from a publication strategy.

Information generated using this licence is maintained in a long-term, secure company database, that is continually available to other internal company researchers. Therefore, data will be recoverable in the future, even after likely project and personnel changes, and the information will be a valuable future resource to reduce the need to repeat and re-establish expertise in a field of research.

Species and numbers of animals expected to be used

- Mice: 13,500
- Rats: 2350

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

It is the aim of this project to identify and develop new medicines to treat diseases affecting the immune system. Prior to performing in vivo experimental models under this licence, in silico and in vitro human and animal models are regulary employed to study biological responses and mechanisms related to the human disease. In vitro biological cell based assays are reproducible, reliable, robust and biologically relevant enabling the screening of many compounds to identify those with the desired biological effect for progressing in vivo. Currently, animal models cannot yet be replaced in all pre-clinical research as they are required to provide basic information relating to the function of specific mechanisms and targets, and for assessing the activity of potential new treatments in complex, integrated systems only found in living animals. For most of the proposed work an established immune system is required that is able to respond to a challenge and/or develop disease symptoms, and is suitable to investigate the roles of genes of interest that may be involved in human disease. For rodents, this would typically require animals of at least 8 weeks of age. Animals may be genetically altered resulting in developing

diseases naturally (spontaneous), and/or can be treated (induced) to develop the required response and/or relevant disease symptoms which are similar to those observed in humans. Animal models are essential for providing such conditions, as they present with many similarities regarding the features and characteristics of the human disease they intend to reproduce, therefore there is a high level of translation between species. The recognised similarities validates rodents as highly relevant and extremely valuable model systems for the work proposed in this licence. The animal models used for this work have been developed to be the least severe, and are well characterised and validated to investigate the role and function of mechanisms and pathways involved in immunological and inflammatory changes. The processes involved in disease states are extremely complex, thus new treatments need to be tested in a whole living animal to ensure effects produced on immune responses are accurately predicted. Within this project licence, it is proposed to use adult rats and mice with mature immune systems to test the effectiveness of new treatments.

Typically, what will be done to an animal used in your project?

An animal used under this project licence will typically experience the following.

Protocol 1 (Tissue supply)- Animals will not undergo any disease induction, and will be used to supply tissues and/or fluids to support work performed in culture (e.g. ex vivo). Under this protocol, animals will be humanely killed to allow blood and/or tissues to be collected. As part of this protocol, animals may receive either a single or repeat course of drug treatment prior to collection of samples. Drug levels and/or markers of treatment effects in the blood may also be measured. At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 2 (Mechanistic challenges) - Animals will be injected (typically on a single occasion) with an inflammatory agent (e.g. LPS) to produce an inflammatory/immune response that could lead to moderate suffering and last up to 7 days. An animal could receive either a single or repeat course of drug treatment throughout this duration. The effect of any given drug treatment on the inflammatory/immune response produced will be assessed by measuring drug levels and/or markers of treatment effects (i.e. cytokines) in the blood. Animals will be weighed and general health assessed daily throughout the study duration. At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 3 (Immune responses) - Animals will be injected (typically on a single occasion) with an antigen (e.g. KLH) to produce a primary immunisation response. A second injection (typically on a single occasion) may be provided to produce a secondary immunisation response (e.g. DTH) that could lead to moderate suffering and typically last up to 14 days. Animals could receive either a single or repeat course of drug treatment during this duration. The effect of any given drug treatment on the inflammatory/immune response produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. antibodies) in the blood, measurement of tissue responses (i.e. ear swelling), and/or via imaging capabilities (e.g. MRI). Animals will be weighed and general health assessed daily throughout the study duration. At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 4 (Mouse arthritis) - Animals will receive a single injection of an initial sensitising substance (e.g. collagen/antibody cocktail) followed by a further single injection (boost) of a secondary inflammatory agent (e.g. LPS) up to 1 week later to induce arthritis. Animals will start to show signs of

joint swelling in all paws within 2-3 days following the second injection which could last up to 3 weeks which could lead to moderate suffering. Animals will be weighed and general health assessed daily throughout the study duration. Animals are likely to receive either a single or repeat course of treatment during this duration. The effect of any given treatment on the arthritis produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. antibodies) in the blood, physical observations (i.e. paw swelling) and/or via imaging capabilities (e.g. MRI). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 5 (Rat arthritis) - Animals will receive an initial single injection of type II bovine collagen usually combined with an adjuvant (e.g. IFA) followed by a further single injection (boost) 7 days later of type II collagen combined with an inflammatory agent (e.g. LPS) to induce arthritis. Animals will start to show signs of joint swelling in the hind paws within 5-7 days following the second injection which continues until day 21 post initial collagen injection that could lead to severe suffering. Animals will be weighed and general health assessed daily throughout the study duration. Animals are likely to receive either a single or repeat course of drug treatment administered during this duration. The effect of any given drug treatment on the arthritis produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. antibodies) in the blood, physical observations (i.e. clinical scores, paw swelling) and/or via imaging capabilities (e.g. bio-luminescence). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 6 (Mouse colitis) - Animals will receive either a single injection of T cells (adoptive transfer model), or antibody (e.g. aCD40), or will be administered with a chemical substance (e.g. DSS) in the drinking water, to induce colitis. Animals will typically start to show symptoms of colitis (e.g. body weight loss) 2-3 weeks following T cell transfer or 2-4 days following either antibody or chemical administration. Animals will be monitored daily and general health assessed. Animals will be weighed every other day until disease symptoms are observed, then every day thereafter. Animals could receive either a single or repeat course of drug treatment during this duration. The effect of any given drug treatment on the colitis produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. proteins) in the blood and/or faeces, or physical observations (i.e. endoscopy). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 7 (Mouse Lupus) - Animals that are genetically altered (e.g. mouse strains NZB/W F1 and MRL/lpr)) will either spontaneously develop lupus symptoms, or animals will have a single injection of a disease accelerator (e.g. pristane) or an immune trigger such as an adeno-virus (e.g. Ad-IFN) to initiate lupus development. Genetically susceptible mice and those challenged with an accelerator will typically start to show lupus symptoms (e.g. protein in the urine, enlarged lymph nodes, enlarged spleen, arthritis) from 2-6 months of age, while those challenged with adeno-virus will show symptoms from 6-12 weeks of age. Animals will be weighed and general health assessed daily throughout the study. Animals could receive either a single or repeat course of drug treatment during this duration. The effect of any given drug treatment on the lupus produced will be assessed by measuring drug levels and/or markers of the treatments effects (e.g. auto-antibodies) in the blood, and/or via imaging capabilities (e.g. MRI). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for furtheranalysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals exposed to an inflammatory/immune challenge agent (protocols 2 and 3) are likely to experience short lasting local or systemic inflammatory and/or immune responses, although certain models may be associated with weight loss (e.g. 5 - 10% of highest achieved body weight) lasting for a few hours or several days after. Additionally, adverse effects such as hunched posture, piloerection, subdued responsiveness, ocular-nasal discharge and diarrhoea may be observed, although these usually subside and animals recover within a few hours/days depending on the challenge agent and model used.

Animals that develop arthritis (protocols 4 and 5) may display signs of weight loss (e.g. 5 - 15% of highest achieved body weight), hind limb swelling and/or loss of weight bearing, reduced mobility, scabbing at the injection site, and may experience some degree of pain during the development (e.g. 5-7 days following boost) and maintenance (e.g. up to 21 days from initial collagen injection) of arthritis symptoms.

Animals that develop colitis (Protocol 6) may display signs of weight loss (e.g. 5 - 20% of highest achieved body weight), develop diarrhoea, hunched posture, blood in faeces (e.g. DSS model), swelling and reddening of skin, especially around the eyes, and may experience some degree of pain

Animals that develop Lupus (protocol 7) may display signs of weight loss (e.g. 5 - 10% of highest achieved body weight), develop protein in the urine, enlarged spleen, enlarged lymph nodes, skin rash, arthritis, and may experience some degree of pain.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Experience during work under previous licences indicates that at least 25% of animals are expected to show no more than mild effects following treatment. Up to 60% of animals will likely, experience a cumulative moderate severity, or at least a period of moderate severity at some point during experiments. Up to 15% of animals will likely, experience a cumulative severe severity (e.g. rat CIA model).

Protocol 1 is non-recovery (100% of animals), Protocols 2-4 will be moderate (40% of animals), Protocol 5 will be severe (up to 40% of animals may enter severe category, but all attempts will be made to work within the moderate severity), protocol 6 is moderate (40% of animals) and protocol 7 is moderate (up to 100% of animals have the potential to enter moderate severity).

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 08 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this programme of work is to identify and develop new treatments for extremely complex human conditions that affect many organs and/or systems in the body that have many different causes (e.g. hormonal, genetic, cellular). While it is possible to reproduce some aspects of these systems and study certain aspects of these functions in isolation (e.g. in vitro), the complex processes and interactions that work in different ways and at different times, either alone or together, cannot be completely and accurately replicated outside of the whole animal. The immune system is a very complex, highly coordinated network of specialised organs and cells that protect the body from potential harmful substances (e.g. bacteria, virus, infection), which can respond quickly and efficiently (i.e. immune response) to eliminate such harms. These responses involve several cell types (e.g. white blood cells) and signalling proteins (e.g. cytokines), and consist of a sequence of events that are common across mammalian species, and hence results from experiments carried out in rodents can be compared and translated to humans. Responses are coordinated to identify potential disease causing organisms (e.g. a virus or other pathogen) and/or damaged tissue, and act to alert and recruit other cells and signalling molecules and/or activate processes that can lead to structural changes in tissues and organs. An inefficient or malfunctioning immune system can result in the body mistakenly begins attacking its own healthy cells, tissues and organs leading to autoimmune diseases that may be restricted to certain organs (e.g. Lupus) or involve a particular tissue in different places (e.g. rheumatoid arthritis).

Additionally, investigations into disease states that are the result of altered or mis-functioning immune mechanisms, or investigations into highly organised body systems require the use of mature functioning tissue(s) that cannot currently be fully replicated, grown or kept alive outside animals (ex vivo). Rheumatoid arthritis (RA) is an example of an autoimmune disease primarily affecting joints of the hands and feet resulting in inflammation, pain and reduced function. The underlying cause of the disease is unclear, however due to the nature of the immune response and the structure of the joints/tissues involved, it remains too complex to replicate the cellular responses, tissue damage, symptoms and course of the disease in a plastic tube. Whilst a wide range of information from isolated cell systems is generated as part of the initial investigatory process to further increase our understanding of the basic mechanisms and how potential treatments target or affect many functions, understanding the integrated response in a whole animal with a physiology that is common with humans is vital to direct potential medicine progression to human clinical trials.

Which non-animal alternatives did you consider for use in this project?

Various in silico and in vitro assays are used to predict and/or investigate whether a novel therapy can directly effect isolated cellular processes involved in immune diseases. For example, cells and/or tissues may be isolated from naïve, diseased or challenged animals for use in biological assays. Cell

populations may be isolated from tissues and cultured as cell suspensions, or tissue pieces may be cultured in plastic tubes as explants or organoids. Models using types of isolated human and animal cells have been developed and widely published in the scientific literature, and are being increasingly used as part of the development of new medicines. Currently, the vast majority of these models use flat cultures of cells growing on plastic that have a limited level of complexity, and are only capable of addressing specific questions. The continued development of organoids (e.g. intestinal epithelial system) and three-dimensional (3D) modelling systems (e.g. gut-on-a-chip) have enabled some of the challenges of investigating the cellular interactions to be addressed, however, the full level of complexity required to replicate the human disease situation are yet to be achieved.

Why were they not suitable?

Despite much progress over the last few years, the three dimensional (3D) systems such as gut-on-a-chip and joint-on-a-chip are limited in their availability and they do not model the complex processes of the immune system. Immune responses involve the integration of genetic, molecular and cellular factors, across multiple systems. Additionally, systems using cells are not yet adequately able to model potential drug absorption, distribution, metabolism and elimination (ADME) properties throughout the body resulting from the administration of a therapeutic agent passing through organs such as gut and liver to enable delivery to the target organ/tissue. Thus, these non-animal systems are not yet fully characterised and/or validated to provide confidence in the clinical relevance of data generated from using them. While these models provide great value in predicting the potential effects of a novel therapy, Given this level of complexity, there are currently no suitable alternatives to assess the efficacy of a novel therapy on the ability to produce a desired or intended result, without the biological changes seen in the animal models.

A retrospective assessment of replacement will be due by 08 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from yourexperience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimated number of animal that are likely to be used over a five year period are based on the experience of related work and projects previously supported under licence authority within the company. There is an anticipated steady rate of projects likely to require information that cannot be gained from non-animal alternatives. Also considered in these estimates is the likelihood of changes to research priorities as a result of continuing scientific advancements in many areas of science. Because

we are familiar with the types of models that are likely to be used and know the resources that we have available we can estimate the number and type of studies that will likely be required over the life cycle of a licence.

We use accepted statistical principles based on the main readouts from each model together with knowledge of the variability those readouts to inform on animal numbers required per type of study to produce statistically useful information.

The number of animals to be used under this licence has been estimated through previous experience and the anticipated requirement, over a five year period, to identify and develop novel therapies/therapeutic agents for the treatment of autoimmune and inflammatory diseases. The animal models used under this licence have been developed and optimised with statistical input to ensure they have sufficiently powered robust and reproducible endpoints, using the minimum number of animals to make informed decisions.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

All experimental work is planned with the input of statisticians to ensure that experiments provide high quality data using the minimum number of animals. Experimental treatment group size will either be based on existing data, or small trial (pilot) studies will be carried out to establish the variation of the biological system and associated readouts being investigated. Understanding the variation and what constitutes a meaningful biological response to a treatment allows a statistician to calculate treatment group sizes that are used to ensure that statistically meaningful comparisons can be made between treatment and control groups. These design principles aim to reduce the possibility of experiments not generating decision making data, potentially resulting in repeating work and hence using more animals.

In addition to statistical support, all studies conducted under this licence will undergo internal peer review in order to ensure that all aspects of experimental design are suitable for the study being proposed.

Where the biologic effect of a new therapeutic agent or control treatment is being tested, the blood or tissue levels of that substance and the associated biological response will be measured from the same animal. This allows the direct comparison of treatment levels and treatment effects to be made, and reduces the number of animals required overall. Animals will be randomly assigned to experimental groups using a random number generation system. This will reduce bias in a design that could compromise the value of generated data and potentially lead to more animals having to be used if work were to be repeated. Additionally, for subjective readouts that require a person to make a judgement, bias in data interpretation will be avoided by using experimental blinding. Thus, those involved in making those assessments will not be influenced in the interpretation of readouts by knowing which treatment had been given to animals.

These robust study design measures will maximise the likelihood of generating non-biased experimental results, and limit the number of animals required to generate high quality decision making data.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Before performing any work under this project, cell assays (e.g. in vitro) will be performed to determine the activity of the test agent on the relevant mechanisms. Where a new model is required or an additional endpoint is to be implemented within an existing model, initial pilot studies will be undertaken to optimise the conditions and measures will be taken to ensure the maximum level of information (e.g. via serial sampling) is obtained using the least number of animals. Model performance will be continuously monitored and opportunities to reduce variability will be implemented with help from a statistician. To reduce variation in biological readouts we will ensure that our facilities provide a constant optimal environment suitable for the species, and the number of personnel involved in making any subjective experimental measures will be minimised. By controlling variation in biological readouts, we aim to ensure that the minimum possible animal group sizes are used to achieve the scientific objectives of the study.

A retrospective assessment of reduction will be due by 08 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will take a staged approach to studies, minimising the risks to scientific objectives and animals:

Blood and tissue supply (Protocol 1)

Aspects related to understanding particular mechanisms and/or process involved in inflammatory and immunological processes are evaluated initially through cell assays performed in tissues provided from non-recovery studies (Protocol 1). Additionally, materials (e.g. T cells) will also be provided for other in vivo models under this licence (e.g. adoptive T cell transfer model of IBD).

Acute challenge models (Protocol 2)

Following confirmation and validation of a mechanism and/or target in cell assays, initial in vivo validation will be performed in an appropriate challenge model (Protocol 2). These models display certain features of human autoimmune and inflammatory diseases, however they do not develop

disease-like symptoms and have a short duration, causing less harm than the disease models. They are used to provide further understand of the specific effects of new targets and/or mechanisms in vivo, thus limiting the number of compounds tested in the disease models.

Immune response models (Protocol 3)

Once initial validation of the mechanism and/or target has been determined, further evaluation may be performed in immune response models (Protocol 3) to assess their effects on specific immune responses related to a particular disease, without the need to induce a chronic disease state and the associated harms to animals. The selected models will allow us to explore how altering certain processes and/or functions with effective treatments can modify the immune responses being produced. The information provided form these models will provide further understanding into the mechanism of action of the target, and will provide evidence to support progression into more complex, disease-like models.

Arthritis models (Protocols 4 and 5)

The selected arthritis models (Protocols 4 and 5) represent many of the features of rheumatoid arthritis (RA) and are extremely useful for investigating mechanisms and processes involved. However, no single model reproduces the human disease exactly. Therefore, prior knowledge of the targets and mechanisms being investigated are essential for model selection to assess effective treatments. The mouse CAIA model (Protocol 4) will be the preferred option due to the quick onset of disease symptoms, produced over a duration of no more than 12 days, causing the least harm to the animals. Only in specific instances where the testing of a particular mechanism and/or target is necessary, will the 21 day rat model be considered. This approach will provide all the required data while preventing prolonged harm to the animals.

Mouse colitis models (Protocol 6)

The mouse models of colitis represent many of the common characteristics of human inflammatory bowel diseases (IBD). While no single model reproduces the human disease exactly, the selected models are valuable tools for assessing effective treatments on the mechanisms and processes involved. The DSS model resembles key features of ulcerative colitis (UC), has very quick onset of symptoms produced over a short duration of 7 days, that resolves quickly, providing a simple, robust model. The adoptive T cell transfer model assesses long term colitis that requires a longer duration of 6 weeks for the required disease symptoms to develop, however this provides a wider, more detailed disease assessment to test effective therapies on specific targets and/or mechanisms involved. The approach taken will provide all the required data while preventing prolonged harm to the animals.

Mouse SLE models (Protocol 7)

Different mouse models for Spontaneous Lupus Erythematosus (SLE) will be used (Protocol 7), as a single model alone does not represent all the disease characteristics seen in human disease, required to assess effective treatments. The mouse strains we will investigate, develop mild symptoms of lupus spontaneously at approximately 3 and 5 months of age. The disease onsets can be accelerated and aligned to levels of proteinuria similar to the human disease, by using inflammatory accelerators, allowing us to minimise the period of time for disease development, thereby reducing the cumulative

harm of disease and treatment period. Prior knowledge of the target and/or mechanism to be tested is essential for appropriate model selection and prevent prolonged harm to the animals.

New models and Biostatistics

When new models and/or modifications to existing models are required, pilot studies will be performed to ensure the animal model is as refined as possible. The methods employed in each animal model will be suitably refined to ensure the animals are exposed to the least harm to achieve the scientific objectives.

Statistical support will be obtained at all stages of running an animal study, to ensure the least number of animals are exposed to harms to provide the required scientific data. Literature will be regularly assessed to ensure the most up to date models, methods and refinements are used.

Harms associated with each disease model have been minimised by reducing the duration of the model and/or disease and by setting clear humane and experimental endpoints. The humane endpoints consider all aspects of the physical signs or symptoms associated with the particular model and/or disease.

Why can’t you use animals that are less sentient?

Autoimmune and inflammatory diseases are extremely complex conditions affecting single or multiple organs of the body, often involving several systems simultaneously. Rodents are a relevant species as they provide many similarities with the mechanisms, systems and processes involved in human diseases that are not known to be active in less sentient species. To undertake the work proposed under this licence, rodents must have an immune system that has matured sufficiently that it consists of all the required functioning components. Therefore, this requires animals to be at least 6 weeks of age, but preferably in excess of 8 weeks of age. Due to the duration of the models to be used, it is impractical and unethical to keep animals anaesthetised for the duration of the required procedures and sample collection periods.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The models described within this licence will undergo continual review to ensure they cause minimum harm to animals, while maintaining scientific integrity. For example, the duration of model implementation will be continually reviewed to ensure that the earliest appropriate biological endpoint is used to achieve the scientific objective. Furthermore, the number and frequency of procedures will be kept to the minimum required to answer the scientific question.

For each inflammatory model, animals will be assessed daily and monitoring score sheets will be used to assess the welfare of the animal as the model progresses.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The following is a list of the best practice guidance that we routinely follow.

Percie du Sert N et al. The ARRIVE guidelines 2019: updated guidelines for reporting animal research. BioRxiv. 2019: 703181.

Smith A et al (2018). PREPARE: guidelines for planning animal research and testing. Lab Anim; 52(2):135-141.

Prescott MJ, Lidster K (2017) Improving the quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156.

Review of harm-benefit analysis in the use of animals in research. Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group chaired by Professor Gail Davies (Nov 2017).

Review of harm-benefit analysis in the use of animals in research - Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group chaired by Professor Gail Davies Nov 2017

NC3R's - Responsibility in the use of animals in bioscience research: Expectations of the major research council and charitable funding bodies

LASA - Guiding principles on good practice for Animal Welfare and Ethical Review Bodies Sep 2015

Guidance on the operation of the Animals (Scientific Procedures) Act 1986. (Home Office 2014).

Kilkenny C et al (2010). Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6).

Diehl KH et al., (2001) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes: Journal of Applied Toxicology 21, 15–23

Morton DB et al., (1993) Removal of blood from laboratory mammals and birds Laboratory Animals 27, 1-22

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in the 3Rs through regular referral to the NC3R's website, as well as other published literature. Furthermore, the establishment Named Information Officer (NIO) will facilitate the dissemination information in relation to any such advances. In accordance with any updates, we will review and revise the protocols within this licence to ensure they have been adequately considered, and where applicable, applied.

A retrospective assessment of refinement will be due by 08 January 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

10. Bacterial adaptations to host environments

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Respiratory tract, Bacterial infection, Therapeutics, Vaccines

Animal types	Life stages
Mice	adult, juvenile, embryo, neonate, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To be able to cause disease, bacteria must be able to thrive within their host. We aim to understand how different aspects of the host environment, such as the available nutrients and immune defences, can shape the processes of bacterial adaptation and evolution. We aim to harness the information gained to develop new therapies and vaccines for the treatment or prevention of bacterial disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Antimicrobial resistance is an accelerating public health issue. We urgently need to identify new ways to treat drug-resistant bacterial infections. Understanding how bacteria capitalise on the opportunities provided by the host environment, and how they overcome the challenges presented, can help us to develop new strategies to combat infection. These might include developing drugs that prevent bacteria from utilising host resources, or therapies designed to promote aspects of immune defence that are key to eliminating bacterial pathogens.

What outputs do you think you will see at the end of this project?

This project will provide the following outputs:

- New understanding of microbial determinants of colonisation and virulence for important bacterial pathogens of the human respiratory tract.
- New information regarding how bacteria sense and respond to their host environment.
- Multiple research publications in peer-reviewed journals. We have a strong publication record as a lab and aim to publish from all our funded studies (typically 2-4 publications per year).

  Trained scientists. The skills learned from work on this project will be invaluable for the future research careers of the staff and students involved.

  In the long term, we would hope to generate novel therapeutics and/or vaccines for the treatment/prevention of bacterial diseases.

Who or what will benefit from these outputs, and how?

In the short term, the major beneficiaries will be the staff and students employed on the project, who will benefit from high quality training and exposure to a range of research methods. Both they, and the research community more broadly, will benefit from access to research findings in the form of published research papers that will stimulate further research. The research community will also benefit, in the short to medium term, from access to new experimental techniques that we will develop during this project, including those for novel experimental evolution systems and bespoke bacterial growth media that replicates conditions of the airways. In the long term, the public will benefit from access to new therapeutics or vaccines. In particular, people with cystic fibrosis would benefit from the development of novel therapeutics designed to increase the effectiveness of antibiotic treatment of infection.

How will you look to maximise the outputs of this work?

We aim to publish in open access journals to ensure maximum reach of our research and we also use pre-print servers (e.g. bioRxiv) to make our findings available at an early stage. We will publish a methodology papers to disseminate our work on developing novel culture media, and any data that is not used in primary research publications will be uploaded to Wellcome Open Research as a dataset. All our high-impact publications are accompanied by press releases to make the wider public aware of significant developments.

We have a number of active national and international collaborations and these will likely develop over the course of the project. For example, we have contacts in the pharmaceutical industry with whom we may wish to collaborate on the drug development aspects of the planned work.

Species and numbers of animals expected to be used

- Mice: 6800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We study bacteria that cause disease in the respiratory tract. We are interested in how the environmental conditions of the upper airways (the nose and sinuses) and the lower airways (the lungs) can influence bacterial evolution and determine the outcomes of disease. Of the animals with a respiratory tract sufficiently similar to that of humans, mice are the simples and therefore the most appropriate for our purposes. For most of our experiments, we use adult mice, as the disease course of an adult mouse infected with the bacterial pathogens that we use is very similar to that seen in humans. Occasionally, we use juvenile mice. Juveniles are useful for studies aimed at developing new vaccines, as we typically aim to vaccinate children and want to mimic that situation as best as possible in our models.

Typically, what will be done to an animal used in your project?

Most of the protocols in this licence aim to reproduce the clinical features of bacterial respiratory infection. These include asymptomatic bacterial carriage in the upper airways, pneumonia, chronic (long-term) lung infection and sepsis (infection of the bloodstream). These experiments range in length from 24-72 hours for a pneumonia or sepsis experiment up to >1 month for an upper airway carriage or chronic lung infection experiment.

In most cases, we can establish bacterial infection of the airways in mice by simply administering some bacteria suspended in saline to the nose of the animal and allowing the liquid to be inhaled. For sepsis, we administer the bacteria directly into the bloodstream by injection of a tail vein. For the induction of chronic lung infection, we embed bacteria into a jelly-like substance called agarose and then inject the agarose (as microscopic beads) directly into the airways. This requires surgery performed under anaesthesia.

Infected mice are monitored for signs of disease or may receive additional procedures. These would typically be either those designed to treat the infection (for example, administering an antibiotic or a novel drug) or those designed to help us understand how bacteria cause disease. An example of the latter would be administering substances that alter an aspect of host immunity, to see whether this alters the outcome of infection. Compounds are usually given in drinking water, are inhaled or are injected into the skin, muscles or blood.

We sometimes use mice that lack a particular gene (knock-out mice) or that express a new gene (transgenics) in order to study particular processes that occur during infection.

In addition to our work on understanding the processes that underpin disease, we also aim to develop new treatments and vaccines. Therefore, some mice undergo treatment with novel drugs and some are given novel vaccine formulations to determine if they provide protection against subsequent infection.

What are the expected impacts and/or adverse effects for the animals during your project?

Once infected with a bacterial pathogen, mice typically either remain healthy or slowly start to develop the characteristic signs of bacterial respiratory disease or sepsis. These include a loss of energy, mild weight loss and a hunched appearance. Once clinical signs are observed, we see either deterioration over 1-3 days, maintenance of mild symptoms for around 7-10 days or else rapid recovery. Weight loss is rare, but can occur, particularly in some of the longer term infection models.

Beyond the infection itself, the substances we administer to the animals and the routes and doses of administration do not typically cause lasting harm. They may alleviate the clinical signs of the infection or may hasten decline, but in either case, we have well defined humane endpoints and so terminate studies before animals suffer unduly.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

None of the protocols under this licence have a severity level higher than moderate. Our work on upper airways bacterial carriage (approximately 1/3 of our total animal usage) is typically mild in severity, although a small proportion of mice (<10%) may go on to develop symptomatic disease. Our work on pneumonia (1/3 of total animal usage) would be classified as moderate, with mice developing symptomatic infection but our humane endpoints preventing excessive suffering. Of all our models, the chronic lung infection model has the most adverse events, with mice experiencing a surgical procedure and mild/moderate weight loss in the days following. However, we use low numbers of animals under this protocol and published guidelines have refined the protocol to minimise adverse events. When performed correctly, mice under this protocol recover their weight and are alleviated of clinical signs within ~10 days of onset of infection.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Mammalian physiology, anatomy and immune responses impact the nature of host-pathogen interactions in ways that can't be reproduced in a laboratory. This project licence is focussed on pathogen adaptations to host environments and the harnessing of that information to develop novel therapeutics and vaccines. Although we attempt to replicate the environment of the respiratory tract in the lab, that data generated are only useful if we can validate their relevance using animal models.

Which non-animal alternatives did you consider for use in this project?

In vitro systems (cell culture or bacterial growth media) offer some opportunities to replace animal use. We never use animals for early therapeutic screening and we use culture systems to study effects of individual host factors on bacterial adaptation and evolution. Performing preliminary work and basic mechanistic studies in vitro ensures animals are only used when alternatives are unsuitable for the research question being addressed.

Alternative infection models, that do not use rodents, include invertebrate species (for example, wax moth larvae) and human experimental models for the study of pneumococcal upper respiratory tract carriage. Human experimental systems are not appropriate for much of the work we undertake, such as disease studies or evolution experiments where bacteria may change in unpredictable ways over time. We use invertebrates for virulence screening and initial drug toxicity testing, before moving to mouse models with selected bacterial isolates or promising drug/vaccine candidates.

Why were they not suitable?

Non-animal systems can replicate many important aspects of the host but can not reproduce every feature. When studying the processes of bacterial evolution, it is important to capture the full picture of the host environment as even small changes in environmental conditions can have profound influences on the way in which the microbes evolve. Similarly, if we aim to study key interactions between a pathogen and its host, we must consider that the interaction takes places in a host-specific context and that the outcome of the interaction may not be the same in a laboratory environment as it would be during an active infection.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

I have been working in this research area for 10 years and have experience of the mouse numbers required to complete comparable projects. We are able to accurately determine expected effect sizes (with variance) of interventions from previous work and we know the frequency at which adverse effects can occur in our protocols. The number of animals required has been determined based on our currently funded studies and the programme of work associated with each.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Our funded studies are peer reviewed, including statistical analysis plans. We use the NC3Rs experimental design assistant for study design, powering our experiments to achieve a define primary outcome measure. Sample size calculations use estimates of effect size from preliminary data or published work. Common experimental designs include time-point analyses and survival studies. In the latter, we monitor disease progression using a sensitive disease scoring system, recording physical/behavioural changes in the animal and using time to progress to humane practical endpoints as a proxy for survival time. Kaplan-Meier survival analysis compares virulence of bacterial strains or treatment and control groups.

In time-point analyses, we assess microbe and host responses over an infection time-course. We first determine the key timeframe where the host-pathogen interaction under investigation can be studied, to reduce the number of time-points/mice needed in subsequent experiments. For acute infection, innate immune responses peak between 12 and 24 hours post-infection, and we avoid earlier or later time points unless there's a compelling need to include them. In chronic lung infection with *Pseudomonas aeruginosa* or URT carriage with *Streptococcus pneumoniae*, key interactions occur later and alternative time-points are prioritised. Longitudinal data are analysed by two-way ANOVA with correction for multiple comparisons.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The majority of animals used are purchased from external suppliers and we purchase only what is needed. When we breed mice, we use both sexes, where possible. Pilot studies are used to determine effective treatment doses or to identify appropriate time points for analysis. Our team work closely with departmental colleagues to share resources and we regularly provide mouse tissue for others when we don't require it ourselves.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use mouse models of infection with human bacterial pathogens. These are predominantly respiratory infection models, although we occasionally use sepsis (blood infection) models when assessing therapeutic or vaccine efficacy. The procedures for induction of respiratory infection are generally minimally invasive, with bacteria in suspension applied to the external surfaces of the nostrils with a pipette, before natural inhalation by the animal. Light anaesthesia is usually administered, to minimise stress.

Following infection, mice develop either upper respiratory tract (URT) infection, which is usually asymptomatic, or lower respiratory tract (LRT) infection, which induces pneumonia +/- sepsis. We can control this process by variation of dose, volume and bacterial strain. We never induce LRT infection unless necessary. If we want to study colonisation, for example, we induce URT infection, as this model causes the least pain and distress.

Occasionally, we administer bacteria embedded in agar beads directly into the lungs of mice. This is a surgical procedure and is associated with a higher frequency of adverse events than our other models. For this reason, it is used sparingly and only where alternative models are unsuitable. The reason for doing so is that it mimics the high-density lung infection with *P. aeruginosa* that is often seen in people with cystic fibrosis. Alternative animal models do not reproduce this long-term infection with large numbers of bacteria.

In the infection models described above, we may treat animals with antibiotics or a novel therapeutic, or else we might promote or inhibit certain host or pathogen signalling pathways, to study their function. When this is done, we first determine appropriate timing and dosing of the intervention, to minimise adverse outcomes for the animals whilst ensuring we achieve the desired effect of the intervention.

Why can't you use animals that are less sentient?

This project investigates the effects of the respiratory tract environment on bacterial pathogen interactions with their host. Of the available model organisms with a respiratory tract similar to that of humans, mice are the simplest. We have to use adult mice for these experiments as the course of infection mimics human disease more closely than when juvenile animals are used. It is impossible to study microbial interactions over an infection time course in terminally anaesthetised animals.

The rationale for testing pathogen virulence, and drug/vaccine formulations in mouse models is well established and widely recognised as essential, when supported by appropriate prior in vitro studies. Use of mice as a model system for experimental evolution of microbes is less common, but we and others have demonstrated that it has advantages over in vitro systems.

Adult mice are the most appropriate choice for most of the work to be conducted under this licence. Disease progresses rapidly in infant mice and does not adequately reflect human infection. Aged mice are used widely in infection studies, but are not required for the experimental questions being addressed here. We will use juvenile animals in immunisation studies, as many vaccines are administered to humans in infancy.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I have worked with mouse models of bacterial infection for 10 years, over which time we have developed robust infection models, whereby mice progress through well-characterised, reproducible disease signs following infection. Hunching and pilo-erection are the first signs to develop and mice are monitored with increasing frequency as they slowly progress to a disease stage where they present with weight loss or behavioural change (reduced energy, minor changes in respiratory rate). We have defined endpoints that allow us to collect valuable information without undue suffering to the animal. The scoring system is sufficiently sensitive that the end-point is rarely missed.

Most experiments are performed with CD1 (for *S. pneumoniae* infection) or BALB/c (*P. aeruginosa* infection) mice and we can reliably predict time to reach humane endpoints, with some variation dependent on bacterial genotype or drug interventions. In our natural inhalation *P. aeruginosa* model, humane endpoints are rarely reached. Long-lasting, low-density lung infection is established and, after development of mild disease in early infection, mice are asymptomatic from ~48 hrs post-infection onwards.

Where disease progression is harder to predict (e.g. the agar-bead model of *P. aeruginosa* infection) we monitor weight regularly to prevent unnecessary suffering. The agar-bead model of chronic *P. aeruginosa* infection requires surgical procedures. Refinement in this model is based on published guidelines detailing appropriate surgical procedural refinements, choice of anaesthetic agent and post-

operative care. We consult the Named Veterinary Surgeon regarding anaesthetic and analgesic protocols and in questions regarding animal health.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Dosing and administration of agents, and withdrawal of blood, will be conducted according to LASA guidelines. Our disease scoring system was originally based on that of Morton (Morton DB, Nature, 1985, 317(6033):106) but has been updated to include more recent recommendations (Turner PV, Pang DSJ and Lofgren JLS, Comparative Medicine, 2019, 69(6):451). Animal monitoring will also take account of the mouse grimace score (Miller AL and Leach MC, PLoS One 2015, 10(9):e0136000). The agar-bead model was refined by van Heeckeren and Schluchter (van Heeckeren AM and Schluchter MD, Laboratory Animals 2002, 36:291-312), and more recently by Bayes et al (Bayes HK et al, Sci Rep, 2016, 6:35838).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our Biomedical Services team advertise local and national 3Rs meetings, workshops and conferences. I have attended these and encourage my staff and students to do likewise. Important papers, posters and information leaflets are displayed in our animal unit, highlighting important work in 3Rs areas. I have an NC3Rs funded PhD studentship and have strong links with NC3Rs through my work on one of their funding panels. Our NC3Rs regional manager is in regular contact and on hand to provide support. We constantly aim to apply 3Rs principles to our research and work to implement refinements rapidly, as well as exploring alternative model systems in the laboratory.

Home Office

NON-TECHNICAL SUMMARY

11. Beta cell metabolism and Vitamin B3

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

beta cells, vitamin B3, metabolism, metabolic stress, nutraceuticals

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to study how the metabolic stress response of beta cells (insulin producing cells) in the pancreas can be influenced by providing vitamin B3 precursors to increase nicotinamide adenine dinucleotide (NAD) provision.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In order to preserve quality of life and to lower disease risk during the course of life, we need to understand the mechanisms by which nutrients in our diet are used by biochemical processes in cells to maintain health. NAD (nicotinamide adenine dinucleotide) as synthesised from the vitamin B3 intake in the diet, is a compound that takes part in more biochemical reactions than any other vitamin-derived molecule. NAD links the intake of nutrients to the biochemical processes in the cell. We will study how the biochemical processes in beta cells, the cells that produce insulin in the pancreas, are affected by a diet high in fat or by supplementation with vitamin B3. As impaired beta cell function is an underlying cause of diabetes, this project will lead to a better understanding of diabetes and may give rise to new possibilities for its treatment.

What outputs do you think you will see at the end of this project?

This basic research project will lead to a better understanding of how mutations in the genes for NAD biosynthesis in beta cells can influence energy metabolism and glucose/insulin tolerance in mice. The energy metabolism of mice can be measured while the mice live normally in their cages. All these outputs will be obtained on a diet high in saturated fat and compared to a standard diet with lower fat content. The next step will be to study how the negative effects of a high fat diet can possibly be reversed by supplementation with vitamin B3 and its precursors. The effect of these precursors on islet function will be studied. To shine further light on the biochemical function of the beta cell, we will study which of several possible biochemical reactions is actually used in beta cells to produce NAD from vitamin B3 precursors and how these reactions interact with insulin secretion. As glucose is a major substrate for energy metabolism in beta cells, we will perform tracing experiments to study how glucose utilisation in the cell is affected by variations in NAD levels.

Who or what will benefit from these outputs, and how?

All knowledge gained from the project will be shared with the scientific and clinical community and published in peer reviewed journals (output expected during the project). Once the mechanisms of vitamin B3 metabolism on metabolic stress resilience in beta cells have been studied and better understood, the results will inform experiments to study human viable islets, a very rare resource, to examine the effect of vitamin B3 on human islet beta cell function (output expected during or slightly after the project). The long term benefit of the work will be a better understanding of the mechanisms by which dietary nutrients are used by cellular metabolic processes to maintain health. This will open new avenues to reduce metabolic disease risk for example in type 2 diabetes (output expected some 1-5 years after the project).

How will you look to maximise the outputs of this work?

Discoveries as well as negative results will be shared with the wider scientific and clinical communities through presentation at national and international conferences and publication in highly ranked peerreviewed journals. We will also share best practice of new techniques and newly developed protocols with collaborators. We will engage with the public through events organised by the establishment to showcase our research.

Species and numbers of animals expected to be used

- Mice: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use genetically modified mice, which have mutations affecting the NAD metabolism in beta cells in the pancreas. These mutations will allow us to understand how metabolic stress can influence NAD levels in beta cells. Subsequently these mice will be studied to understand how vitamin B3 precursors can be used to ameliorate metabolic stress.
Adult mice of a specific age range (8-12 weeks of age) will be chosen to minimise variability.

Typically, what will be done to an animal used in your project?

Mice with a beta cell specific NRK1 mutation will be fed a high fat diet for 16 weeks to induce metabolic stress. Subsequently their energy metabolism will be assessed in the TSE Phenomaster System (9 days) and their glucose and insulin tolerance will be measured (2 hours each). At the end of the experiment tissues will be collected for further analysis.
Glucose and insulin homeostasis will be studied in for example in whole body NRK1KO mice compared to islet cell specific NRK1 KO mice. To achieve this, glucose, pyruvate and insulin tolerance testing as well as insulin and glucagon measurements will be performed (2 hours each, up to 6 measurements in total).
To reverse the effects of the high fat diet, mice will either be fed (for 16 weeks) or injected with vitamin B3 precursors and the effect of the precursors on glucose and insulin tolerance or hormone levels (glucagon and insulin) will be measured (2 hours each). At the end of the experiment tissues will be collected for further analysis.

To understand the biochemistry of vitamin B3 precursor incorporation into NAD metabolism and their influence on energy metabolism labelled substances will be injected to study their turnover and distribution in the body (up to 1 day). At the end of the experiment tissues will be collected for further analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Glucose/insulin tolerance testing and hormone measurements lead to transient pain during blood sampling (2min) when the initial tail incision is made and when subsequent blood samples are taken from the same incision over a period of up to 3 hours. To ameliorate this, a local anaesthetic will be applied before venesection. If tolerance testing or hormone measurements are repeated, mice will be resting for 2 weeks between measurements. For energy assessment in the TSE Phenomaster System mice have to adapt to the new environment, which might lead to slight weight loss in some animals. To ameliorate this an acclimatisation phase allows the mice to get used to the environment in their original litter groups before they are single housed during the isolation phase. The energy assessment itself is performed by measuring O_2 and CO_2 concentrations non-invasively while the mice live in their cages.
Mice will rest between TSE measurements and tolerance testing for a week. As high fat diet may lead to skin irritation soft bedding material will be used in the cages to prevent skin irritation from high fat diet.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

About 80% of mice will undergo a combination of procedures, like high fat diet, glucose and insulin tolerance testing and energy assessment or vitamin B3 precursor injection followed by glucose and insulin tolerance testing or mice will undergo glucose, insulin tolerance testing as well as hormone measurements. This will be classed as moderate severity.
The remaining 20% of mice will not undergo a combination of procedures and will be classified as mild.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We use non-animal methods to identify and validate the vitamin B3 precursors that can be used to influence glucose and insulin metabolism in beta cells. While these are important studies, these conditions are artificial and cannot give an accurate picture of the effects in different organs and the whole body in mice or humans. Insulin is produced in the pancreas, but effects many different organs like muscle, liver and adipose tissue. Thus we use mouse models to study how vitamin B3 precursors can influence energy and glucose metabolism in different organs. Furthermore in mouse models specific genes of the biosynthetic pathway from vitamin B3 to NAD can be inactivated to examine their effect on biochemical pathways in vivo.
Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace *in vivo* animal use.

Which non-animal alternatives did you consider for use in this project?

In vitro data obtained from cell culture approaches has guided the proposed *in vivo* studies. We always conduct cell culture based experiments to justify the need to use animals.

Why were they not suitable?

Not all questions can be addressed in cell culture, there are no immortal cell lines available to study the complex interaction of alpha and beta cells in the pancreas. Pancreatic islet organoid cultures are still technically challenging.

Reduction

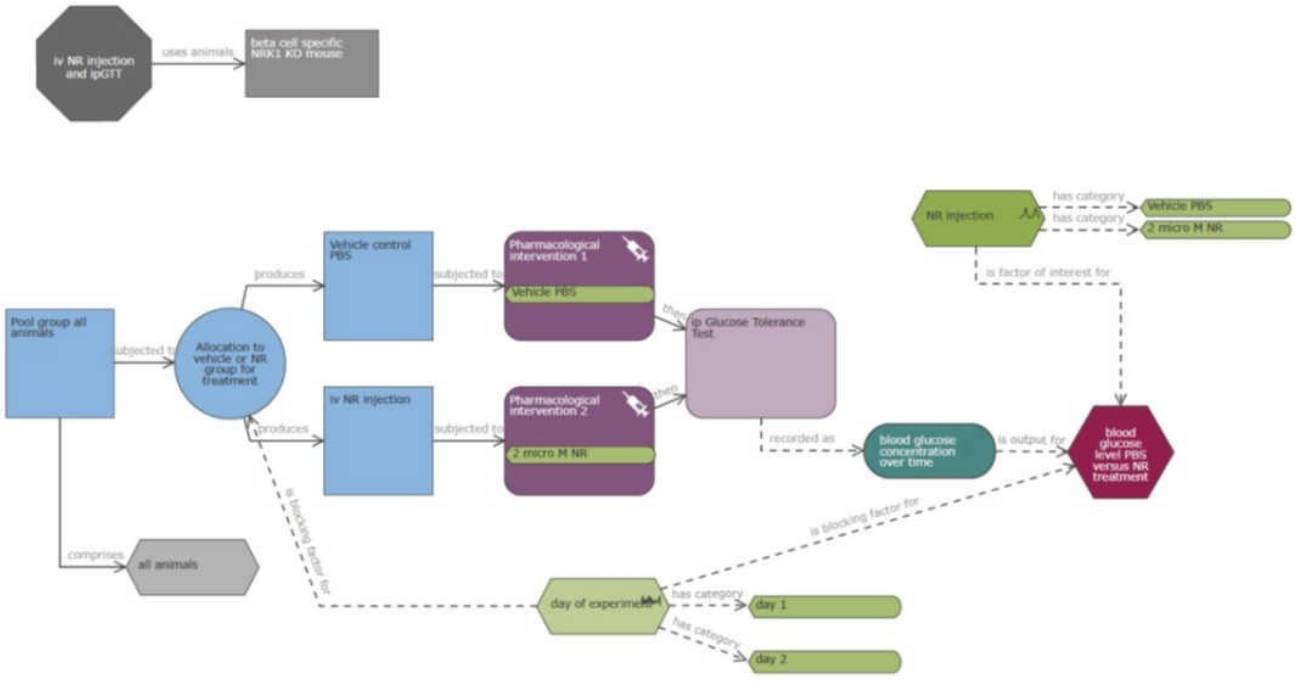
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

When possible we will always use power calculations to determine the number of animals that should be used. For most quantitative experiments, animal cohort size will be calculated via power analysis. Expected effect size will be determined through consultation of the literature, cell culture based *in vitro* analysis or through small pilot experiments when possible.
We have used statistical methods to calculate how many animals we need to get meaningful data, depending on how many different vitamin B3 precursors and how many mutations of the NAD pathway we will look at. We will carefully consider which vitamin B3 precursors will be tested sequentially, for example if nicotinamide riboside has an effect on glucose tolerance, only its building blocks nicotinamide and riboside will be tested as opposed to testing all possible combinations of vitamin B3 precursors in all different mice with mutations of the NAD pathway, thus reducing the number of animals being used.
We aim to characterise the first step of NAD synthesis, using a NRK1 beta cell specific KO mouse. We aim to investigate about 4 different vitamin B3 precursors per year. We will study high fat diet and vitamin B3 supplied in the food as factors that influence glucose and energy metabolism.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used the NC3Rs' Experimental Design Assistant to design experiments (protocol2, example) and taken advice from researchers who have performed similar research projects in the past.



We aim to reduce animal numbers by applying methods to reduce subjective bias, and undertaking appropriate statistical analysis without compromising the scientific objectives. When possible, experiments will involve a factorial design that will maximise the information obtained from a minimal number of animals. Furthermore non-

invasive energy metabolism assessment and subsequent glucose or insulin tolerance testing can be performed on the same animal over a period of time, thus reducing the number of animals used. As importantly, at the end of all *in vivo* experiments, islet cells and several other tissues will be isolated and a large proportion of the scientific output will be generated from these tissues in further *in vitro* experiments for example primary cell culture, protein analysis, metabolomics and genetic studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies to estimate variability and perform power calculations to calculate sample sizes.
Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.
We always strive to generate the most effective breeding strategies to ensure that we obtain mice of the desired genotype with minimal animal wastage. If we are unable to estimate an effect size from our *in vitro* data, the literature, or our collaborators, we will conduct small pilot experiments.

All tissue surplus to requirement will be stored and made available to collaborators within the institution and beyond.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mouse models to study how vitamin B3 and its precursors influence glucose tolerance and energy metabolism while the mice receive an obesogenic diet. The high fat diet used is a very gentle way of generating metabolic stress and allows us to study how metabolic stress can be ameliorated by supplementation of vitamin B3 and its derivatives.
We will use a mouse model with a beta cell specific mutation in vitamin B3 metabolism, this will allow us to study how beta cells in the pancreas are affected by metabolic stress. The mouse model has no harmful phenotype.

To measure the energy metabolism of mice *in vivo* we employ the TSE Phenomaster System. While the mice live in their home cages, CO_2 production, O_2 consumption as well as food and drink intake can be monitored without any disturbance to the animals.
Even though vitamin B3 precursors are well tolerated and usually have no adverse effects, we will use pilot studies to help us to develop dosing regimens for vitamin B3 precursors if administration is not oral to further minimise risk of adverse effects.

Why can’t you use animals that are less sentient?

Mice are the least sentient species that will allow us to achieve our objectives.
We need to study glucose and energy metabolism in adult mice as this is what most closely resembles what occurs in humans. We have chosen to use mice over other less sentient species such as Danio rerio (zebra fish) and Drosophila melanogaster (the fruitfly) as mice and humans share 97.5% of their coding DNA sequences.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Through advice of colleagues performing similar work we are aware of refined methods for glucose and insulin tolerance testing as well as hormone measurements in diabetes research. Treatment doses, duration and route of administration for vitamin B3 precursors will be evaluated in relation to physiological concentrations in the blood, the literature and from previous experience in our group. These measures minimise the possibility of adverse effects.
Animal welfare is a key consideration in all of our protocols and we will be guided by our NACWO and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. Following every experiment and regularly during group meetings we will review our procedures from a welfare standpoint to identify any potential for refinement.
We are fortunate to have excellent colleagues both in Academic groups and within the REDACTED with extensive, relevant animal procedure experience, including diabetes models, from whom we can learn refined techniques. Our team will undergo extensive training on dead animals and require to be authorised by REDACTED before being allowed to perform a procedure on live mice.

Examples: The TSE Phenomaster System allows the quantification of energy metabolism by measuring O_2 and CO_2 concentrations, food and drink intake while the animal is living in an IVC cage without any disturbance. For glucose and insulin tolerance testing a single incision will be made on the tail to take small sequential blood samples rather than sequential cutting for blood sampling from tail vein. We use ultrasensitive ELISA assays for hormone measurements to reduce the blood volume that has to be taken. Cardboard tubes are used for refined handling and body condition charts for humane endpoints.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will constantly consult the literature for experimental design, best practice, and humane endpoints for metabolic and diabetes research in animals. We also consult Simon Bate's book, 'The design and statistical analysis of animal experiments', for experimental design, statistical analysis, and sample size calculations.
Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment (PREPARE: guidelines for planning animal research and testing.
Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.PMID: 28771074).
The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE 2.0 guidelines published by the NC3Rs.
The LASA giudlines: RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)
Jones HRP, Oates J, Trussel l BA (1999) An applied approach to assessment of severity. In: Humane End points in Animal Experiments for Biomedical Research (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 40±7

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will perform literature searches, attend vendor's information sessions, seminars and conferences to find out about new technology and new approaches that we could implement.
We will comply with the ARRIVE guidelines 2.0 (Animal Research: Reporting In Vivo Experiments; www.nc3rs.org.uk/arrive), a NC3Rs-developed checklist of the essential information that should be included in publications reporting animal research.
My Lab group twitter follows the NC3Rs and lab members are subscribed to the NC3Rs newsletter - helping us keep up with advances in the 3Rs which will be discussed an implemented through our lab meetings.

Home Office

NON-TECHNICAL SUMMARY

12. Biocompatibility of medical devices and materials

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

Biocompatibility, irritation, systemic toxicity

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this programme of work is to evaluate some aspects of the safety and biocompatibility of novel materials and devices that will come into contact with body tissues before, during or after a surgical procedure

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

To assess the safety of materials or devices intended for human use to reduce or eliminate any adverse effects.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Over 5-years
Rabbit – 350
Rat – 400
Mouse - 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The nature of the skin irritation test will involve either topical or intra-dermal application; based on the previous *in-vitro* assessments, compounds and materials considered for testing will have very little harm and effect, and as such it is expected that a moderate severity banding is more than appropriate. Expected adverse effects are reddening of the skin at the test site and raising of the skin of approximately 1mm. If an animal scores a 3 at any single observation period; determined by the adverse effects set out previously, this will be brought to the attention of NVS/NACWO and PPL in order to receive supportive treatment or be humanely killed.
Animals will be killed at the end of the procedure.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Laboratory *(In vitro)* testing cannot fully replicate the in-body (*in-vivo)* physiological conditions required to test the safety of novel devices, therefore animal tests are necessary

Reduction

Explain how you will assure the use of minimum numbers of animals.

The number of animals required for each of the irritation protocols is either clearly defined in established methods detailed in international standards.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Animal suffering will be minimised by proactively refining the materials tested by:

(a) consultation with people with expertise in toxicology, animal welfare and statistics,

(b) Laboratory (in-vitro) tests to refine the biomaterials and hence, reduce the level of pain, distress or lasting harm experienced by the animals.

The species identified and the surgical techniques used are clearly defined in established methods detailed in international standards

Home Office

NON-TECHNICAL SUMMARY

13. Biology of Normal and Malignant Blood Cells

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Leukaemia, Microbiome, Infections

Animal types	Life stages
Mice	adult, embryo, neonate, pregnant, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Acute leukaemia is the major type of cancer in children. Although treatment is now very successful with cure rates of around 90%, the treatment is traumatic and toxic.
Our project aims to model key components of the infection-based mechanism by which childhood leukaemia arises.
Over the years, we have generated a body of data indicating that the most common form of paediatric cancer – childhood acute lymphoblastic leukaemia (ALL) is triggered by an abnormal immune response to common infections (e.g. respiratory infections such as flu).
But this only happens in children who are susceptible by virtue of (i) have acquired, as a developmental accident, a leukaemia-initiating mutation in utero; and (ii) have, paradoxically, a deficit of microbial exposures in the first year of life. The latter, principally in the form of benign or beneficial microbes in the gut, are required to prime the new born immune system for proper networked function.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

These modelling experiments with mice will endorse our longstanding infection-based model for the causation of childhood leukaemia (CL).
This would, in turn, encourage a search for a prophylactic intervention (microbiome-boosting) that would prevent childhood leukaemia.

What outputs do you think you will see at the end of this project?

The research we are undertaking has been many years in the making and has a foundation in the basic biology of leukaemia and in the evolution of the immune system.
As a necessary first step in this ambitious goal, we have established a mouse model of ALL in which development of leukaemia depends upon timing of exposure to common microbes. We will be seeking to establish that recognised gut bacterial species, if administered very early in life to 'clean' mice can protect against ALL. We will also be screening the gut microbiome (via stool collection) of newly diagnosed patients with ALL to see if their microbiomes are, as we anticipate, different (less diverse) than those of 'control' children.
We will publish our results in international journals and present our findings in conferences appropriate for our filed of research.
If our hypothesis is proved correct, this will suggest a potential preventative intervention – boosting of the gut microbiome in infants. This would require population-wide administration, orally we assume, with defined (and safe) bacterial species that prime the immature immune system.

Who or what will benefit from these outputs, and how?

Childhood acute lymphoblastic leukaemia (ALL) is a remarkable success story in oncology as this once fatal cancer now has a 90% cure rate. But, alas, this comes at a price. The treatment is both traumatic for very young patients and their families, non-specific and therefore toxic and damaging. The consequences include both illnesses concurrent with treatment (e.g., infectious complications of immunosuppression) and some longer-term health impacts. It follows that prevention, if it were possible, would be much preferred.
Our previous research has been to uncover the causal mechanism of the disease, substantiate the idea that abnormal immune responses to infection are critical triggers and to highlight the long term (5-10 years hence) prospect for intervention and prevention. Timelines are difficult to predict but we hope that within five years we should be in a position to know if microbiome boosting does indeed prevent ALL in our model system.
In anticipation that it will, we will begin to explore with colleagues what kind of bacterial formulations might be appropriate to trial in infants.
We note that risk factors for childhood ALL are very similar to those identified for childhood type 1 diabetes and allergies. All three of these immune disorders track, internationally, with affluence or development in societies;

the highest incidence rates for all three being in Scandinavia. This raises the exciting possibility of a standard ‘fix’ of prophylactic for all three.

How will you look to maximise the outputs of this work?

This project will produce large datasets, particularly for 16s rRNA sequencing and shotgun metagenomic sequence analysis, using both mouse and human faecal DNA samples. We anticipate that these data will be of significant interest to the broader scientific community and plan to make these datasets accessible at the time of publication. Prior to publication, the data will be stored in line with the data policies of both our institute and will be held on servers that are regularly backed up.

Our research findings will be disseminated via the usual scientific routes and we will also interact, via social media, with patient/parent support groups and interested parents (e.g. Mumsnet).

Species and numbers of animals expected to be used

- Mice: We plan to use 1,700 mice over the course of 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use adult immunodeficient mice to study the ability of leukaemic cells to reconstitute malignant haemopoiesis. It has been demonstrated that this strain of mice represents the best model to support the engraftment and expansion of leukaemic cells of human and murine origin.
We will also use wild type and transgenic mice carrying alterations in various genes that may impact normal/malignant haemopoiesis. These strains of mice will allow us to study how leukaemia develops in different conditions of exposure to infections, to study the role of the microbiome in this process and to test whether a preventative intervention may be effective.

Typically, what will be done to an animal used in your project?

Mice may be irradiated and transplanted with leukaemia cells of human or murine origin via intra-vein or intra-bone injection. Mice will be monitored and periodically blood samples from superficial tail vein will be taken to assess the engraftment and expansion of leukaemic cells. Mice will be culled when a sufficient level of engraftment is proved by laboratory test or as soon as they show any signs of distress. These mice will be monitored for up to 9 months post transplant.
Groups of transgenic mice will be transferred from the 'clean' facility to the 'dirty' one. Some mice will undergo faecal transplant before transfer in order to study a potential prevention mechanism.
Alternatively, some pups born in the 'clean' facility will be fostered by mothers from the 'dirty' facility to study how infections in early in life can boost the immune system and reduce the incidence of leukaemia. All these mice will be monitored for up to 30 months of age and blood samples from superficial tail vein will be periodically taken to assess development of leukaemia. Mice will be culled when the leukaemia onset is proved by laboratory test or as soon as they show any signs of distress. **What are the expected impacts and/or adverse effects for the animals during your project?**

Less then 1% of mice are expected to show signs of radiation sickness. These mice will be observed twice daily in the first few days after irradiation and prophylactic antibiotics may be administered.
No adverse effects are anticipated as a direct result of injections by intravenous route. Intra-bone injection may result in bone pain in the recovery period (48 hours). Analgesics will be administered to reduce the pain caused by injection into bone. Complications from anaesthesia at the doses and for the limited time proposed are very uncommon (<1%).
All our mice will be monitored for development of leukaemia. This includes periodically blood sampling from superficial tail veins. Little and transient pain is likely to affect mice. Blood sampling will allow us to determine the onset of the disease and prevent mice from suffering from typical symptoms of the disease.
Mice showing weight loss exceeding 20% or showing any signs of distress (e.g. dehydration, anorexia and unresponsiveness) will be culled.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most of our experimental mice will suffer a moderate level of severity. Our mice will only be subjected to intravein or intrabone injection (one time) and multiple blood samplings. A percentage of mice might develop leukaemia and therefore show a higher lever of severity. We constantly monitor our mice for the onset and the disease and any signs of distress (such as weight loss, dehydration and fatigue).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

There is, at present, no culture method that can identify and quantify cancer (or leukaemia) stem cell function or any non-animal model system that can recapitulate the development of full-blown leukaemia, in the context of a causal role of infections.

Which non-animal alternatives did you consider for use in this project?

90% of our research on childhood leukaemia has been conducted directly on clinical samples and/or in tissue culture.

Why were they not suitable?

There is no *in vitro* system that can mimic the process of leukaemia formation, driven by infection.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For statistical power analysis, we have used G*power software with the following assumption: α=0.05 (type I error rate) and β=0.2 (type II error rate, with statistical power of 1- β) and used our pilot data with respect to mean and standard deviation to estimate the effect size. This approach will be followed for all *in vivo* experiments to select for the correct mouse sample size.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To estimate correct mouse sample size, we liaised with a team of bioinformaticians with a good background in biostatistics. One person of the team is directly attached to this project and has calculated all the mouse sample sizes.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For both ethical and financial reasons, we plan to use the minimum number of mice necessary. To perform the leukaemia stem cell project and model leukaemia in vivo with human and murine cells, we will use only three mice in each replicate group, the minimum acceptable for biological consistency.
For the studies involving transgenic mice, we will perform a very efficient breeding. We will use both wild type and heterozigous transgenic mice of both sexes, thus reducing the number of breedings needed to complete the project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Sustained leukaemia would lead to distress (and death) but we euthanase all mice early in leukaemia development as soon as there are any signs of distress.
No adverse effects are anticipated (or seen to date) via the initial irradiation of mice or injection of cells. Daily surveillance of the health status of mice and weekly assessment (via tail bleed) of the development of leukaemia ensures culling before the onset of any severe suffering.

Why can’t you use animals that are less sentient?

We use mice as we can generate and develop leukaemia that is similar to that found in children. Our studies cannot be undertaken in a less sentient species such as fish. To study development and prevention of

leukaemia, we need to monitor mice for a long period of time. This cannot be done in mice strictly in an immature life stage or terminally anaesthetised.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Mice will be daily monitored for any signs of distress throughout the duration of the experiments. After carrying out procedures, we will increase the frequency of monitoring. Analgesics will be administered to reduce any bone pain post intrabone injection. Prophylactic antibiotic treatment may be administered after irradiation to reduce adverse effects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All our studies will be conducted in accordance with the NCRI Guidelines on the Welfare of Animals used for experimental neoplasia.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep up to date with any new advances published. We will also keep up to date through our BSU staff. They hold regular meetings with scientists, regularly publish a newsletter where they foster the culture of care and upload on the intranet refresher courses about animal welfare, clinical signs and techniques.

Home Office

NON-TECHNICAL SUMMARY

14. Biology of the Pancreas: embryonic development, adult homeostasis and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words pancreas, diabetes, embryonic

development

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The pancreas is the site of virtually incurable diseases, such as diabetes and pancreatic cancer. An exhaustive understanding of the mechanisms regulating pancreatic cell identity will provide insights into pancreatic diseases and would be fundamental for the establishment of novel therapies of diabetes.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The pancreas is a gland composed of different cellular compartments with distinct metabolic functions. Namely, the so-called acinar and duct cells produce and secrete digestive enzymes that promote nutrient absorption in the gut, while the pancreatic islet cells, also known as islets of Langerhans, produce the hormones (*e.g.* glucagon and insulin) that regulate the levels of sugar in the blood. The endocrine islets consist of mostly insulin-producing beta-cells (60% in humans) and glucagonproducing alpha-cells (30% in humans). The vital functions controlled by the pancreas are reflected in the severity and mortality associated with pancreatic diseases, such as diabetes and pancreatic cancer.

Diabetes is a group of metabolic diseases characterised by high levels of sugar in the blood (hyperglycaemia) and is still a leading cause of mortality and illness worldwide. Clinically diabetes is broadly classified into type 1 diabetes (T1D), which is caused by an autoimmune destruction of the pancreatic beta cells, and Type 2 diabetes (T2D), which is characterised by 1) progressive beta-cell failure in their ability to release enough insulin and keep blood glucose under control; 2) insulin resistance.

Currently, more than 400 million of people have diabetes in the world, the number is estimated to be > 3.5 million in the UK. The incidence of diabetes is increasing at an alarming rate and the current annual cost of diabetes to the NHS is already around £14 billion a year. By 2025, it is estimated that five million people will have diabetes in the UK. Adults with diabetes have a 50% higher risk of death from any cause than adults without diabetes, in addition to risk for myriad complications, such as cardiovascular diseases. However, this growing burden of diabetes has not been met with a comparable expansion in therapeutic options. Indeed, current treatments for diabetes focus on managing the symptoms of the disease rather than curing it. Finding a cure to diabetes represents therefore an important medical challenge with huge clinical and economical potentials.

Clinical transplantation of pancreatic islet cells provided proof-of-principle that reestablishment of an adequate beta-cell mass can restore normal levels of glucose in T1D patients. However, the profound scarcity and poor quality of donor organs restrict islet availability for study and transplantation. To overcome these limitations, significant efforts have been focused in the recent years on developing strategies for directing differentiation of human ESCs or iPSCs or for (re)programming of somatic cells (e.g. hepatocytes) into pancreatic progenitors and beta-like cells that can secrete insulin and are responsive to glucose to a certain extent. These advances have relied upon the understanding of the mechanisms and signalling pathways governing pancreas and islet development. To date, stem-cell derived beta-cells mostly resemble immature cells, which are naturally found in the neonatal pancreas, rather than adult mature pancreatic beta-cells. Stem-cell derived beta-cells display indeed inferior glucose-stimulated insulin secretion as compared to native pancreatic beta-cells. Efforts to provide the best road map for generating fully mature beta-cells remain an area of intense ongoing research activities.

While the challenge of curing T1D centres on the replacement of the destroyed insulin-producing beta cells, in the context of T2D the goal is to achieve preservation of the remaining beta-cell function to prevent the progression of the disease. Recent findings suggested that de-differentiation of mature insulin-producing beta-cells to a progenitor-like state and conversion into other endocrine cell types contribute to beta-cell failure in T2D. Therefore, deciphering the mechanisms that preserve the mature beta-cell state and hamper plasticity will be key towards a better comprehension of T2D pathogenesis. Moreover, fundamental beta-cell biology research will reveal important insights that can be harnessed for better treatment of insulin-dependent T2D patients, for instance for reversing or preventing beta-cell de-differentiation.

Finally, full comprehension of the mechanisms underpinning pancreatic cell differentiation will also be of paramount importance for pancreatic cancer, a devastating malignancy with an extremely poor prognosis and

unmet medical need. De-differentiation of pancreatic exocrine cells and aberrant activation of embryonic signalling pathways are often the initiation events in pancreatic cancer, making developmental regulators therapeutically attractive.
To study differentiation and function of pancreatic cells and use this knowledge to prevent and cure diseases, like diabetes, five important experimental tools are available. First, it is possible to culture cell lines established from mouse pancreatic endocrine tumours (*e.g.* insulinoma), which recapitulate some features of beta-cell differentiation and functions. Second, it is possible to differentiate mouse ESCs or human PSCs into pancreatic progenitors and beta-like cells using complex multistep protocols based on cytokines and soluble molecules, which recapitulate normal pancreatic embryonic development. Third, histological sections from normal embryonic or adult mouse as well as human pancreatic tissue can be examined and used to evaluate the significance of *in vitro* observations. Fourth, it is possible to make transgenic mice and knockout mice in which gene expression is altered either in pancreatic progenitor cells (for example, using the Pdx1 promoter) or in differentiating beta-cells (for example, using the Insulin promoter) or in cells of the surrounding pancreatic microenvironment, which are fundamental for pancreatic beta-cell differentiation (for example, using the Nkx3.2 promoter). Fifth, pancreatic cells that are generated *in vitro* either through differentiation of PSCs or reprogramming of other somatic cell types can be grafted into suitable recipient mice (for example diabetic mice) to evaluate their differentiation potentials. Observations based on the first three experimental approaches inform our decisions to carry out experiments with mice. We also access genomic and transcriptomics datasets, publicly available or from our laboratory (for example, gene mutations and SNPs in human patients), to generate hypotheses about gene networks or pathways that influence pancreatic cell differentiation.
My laboratory carries out experiments on cultured cells as much as possible. However, many biological processes are too complex for a complete recapitulation in a dish. The study of pancreas formation and diabetes involves a number of different cell types and organs making *in vitro* studies unreliable but also providing extreme challenges for the use of model organisms, such as *Xenopus* or zebrafish. For these reasons, it is essential to carry out some experiments on mice.

What outputs do you think you will see at the end of this project?

The work under this licence is expected to provide novel information about pancreas biology and the role of developmental pathways in diseases; this knowledge will be disseminated through publications and presentations at seminars and conferences. Our projects have attracted peer-reviewed project grant support and some of the work has been published demonstrating that the scientific community considers it an area worthy of investigation. Our genetic targets of enquiry have been selected for their direct relevance to human pancreatic diseases. Thus, this information will also generate proof of concept for clinical applications. Moreover, from the *in vivo* mouse model studies, we hope to identify mesenchymal signals and conditions that might improve current protocols for beta-cell generation *in vitro* (*e.g.* in terms of quantity or quality of cells available for future islet transplantation). Finally, one important output is to maximise the value of the mice that we study by sharing tissue with other researchers. We are expecting to generate and study transgenic mice and knockout mice for genes that are relevant to pancreas organ formation, beta-cell differentiation and human diabetes pathogenesis. However, the genes that we study in pancreatic tissues are often active in other embryonic or adult tissues, in particular in the brain. Therefore, we can share tissues from our mouse models with other researchers and mice that are sacrificed in this project licence can foster research on other disease relevant tissues.

Who or what will benefit from these outputs, and how?

Primarily, the data will be used by scientists working in my laboratory and will be made available to collaborators and later to the wider public through open-access publications and repositories. We have previously published in journals, such as *Nature Communications*, *Genes & Development*,
Development, and it is likely that the output from future studies will be placed in similar journals. That will enable users worldwide to access and further utilise the data generated in our licence. Staff working on the project will gain training in use of animals and as a result will have enhanced career opportunities. In the long run, the proposed studies will generate data of translational benefit to islet transplantation, as a therapy for T1D. My

group has links with the Diabetes Research groups and human Islet Transplantation Units in the UK, which will facilitate rapid translation of information from animal studies to changes into clinical practices.

How will you look to maximise the outputs of this work?

We have established collaborations with computational biologists and mathematicians to generate computational models based on experimental data that we generated from our mouse models. The models make predictions about *in vivo* experiments to test, which help us to reduce the number of mice to be used. We will disseminate new knowledge through presentations at conferences and conventional peer-reviewed publications. In addition, we share unpublished data via pre-print servers, such as BioRxiv, publicly available repositories (*e.g.* GEO) and protocol sharing websites (*e.g.* protocol exchanges; protocols.io). To maximise the output of our experiments, we intend also to publish work that yields negative results or is confirmatory via BioRxiv.

Species and numbers of animals expected to be used

- Mice: 22500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice to study the mechanisms underpinning pancreas organ development and functions. These processes can only be studied in whole organisms, because they involve a number of different cell types, making *in vitro* studies insufficient. Also, beta-cell function and blood glucose homeostasis involve complex tissue crosstalk among different tissues within the body, including the pancreas, liver, muscle and fat. The mouse is commonly used as a model system to mimic human diseases and to evaluate potential treatments. Indeed, the mouse is a genetic model system, anatomically and phylogenetically closer to humans than amphibians or fish, being therefore highly suited to study diabetes and to advance therapies toward the cure of diabetes. The objectives of the project are to study pancreatic organ formation at crucial embryonic stages and postnatally to study homeostasis and disease in adult tissues. In particular, between embryonic day (E) 14.5 and birth the different pancreatic cell types differentiate, including the insulin-producing cells, and the organ grows and acquires the well-defined final shape.

Typically, what will be done to an animal used in your project?

Mice may be subject to procedures, such as ear clipping for genotyping, and blood sampling to monitor glucose levels and metabolic parameters. Pregnant females will be treated with agents (*e.g.* Tamoxifen, Doxycyline) to induce transgene expression to knockout pancreatic genes or to label cells for lineage tracing experiments. To limit any adverse effects due to IP injections, we will inject pregnant female mouse using 1 ml syringe and 21G 5/8 needle, and inject between inner thigh and genitals to ensure no organs are punctured nor the amniotic sac and developing embryos physically damaged. Moreover, the number of IP injection in pregnant females will be limited to one and, typically, it will be performed before 12.5 days of gestation.
Similarly, adult mice will typically receive injections of agents, such as Tamoxifen, Doxycyline, labelling agents or AVV vectors for controlling gene expression. The mice may be subject to glucose challenges to further study the glucose metabolism parameters, insulin secretion and predisposition to diabetes. Most experimental observations are completed within 3 weeks to 3 months. However, some adult studies may last 6 to 12 months.

What are the expected impacts and/or adverse effects for the animals during your project?

The main aim of this project is to study pancreas formation and function(s) in a mouse model. No severe adverse effects are expected for the animals during our studies. The introduction of substances into pregnant mice is expected to induce only transient suffering. However, there is a risk (less than 1% in our experience) that the mouse will abort the embryos and the embryos will die.

Gene or mutations that are under investigation in our licence might eventually lead to pancreatic hypoplasia, alteration of pancreatic functions or diabetes. Animals with severe and overt diabetes might develop hyperglycaemia and have side effects, including weight loss and increased urine output. The measurement of body weight and observation of body condition (*e.g.* thin, normal, overweight) acts as a surrogate marker of appetite. The measurement of skin turgor and observation of cage bedding for urine output acts as a surrogate marker of thirst. Therefore, our trained animal staff will monitor these animals on a regular basis (twice daily) and specifically watch out for signs of distress, including changes in appetite, urine output, excessive thirst, dehydration, activity (*e.g.* lethargy or hyperactivity), weight loss, unkempt appearance, abnormal posture, and twitching or trembling. Mice that manifest these complications will be humanely killed by a Schedule 1 method and used for the experiments, which include blood collection at sacrifice and subsequent thorough analysis of the pancreas and other related tissues with histological and molecular biology methods. The expected level of severity in the above circumstances is moderate.
Other unrelated phenotypes, which might be due to spontaneous tumour development with aging or infection, indicative of a mouse that is in pain or experiencing stress (*e.g.* hunched posture, reduction in food and water consumption, a dishevelled coat, subdued behaviour and abnormal breathing) are expected to be rare and transient.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In protocol 1 the severity limit is 'mild' and it is expected that over 80% of mice have no phenotype at all.
In protocol 2-4 the severity limit is 'moderate' and it is expected that 70% of mice will experience this phenotype.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Primary pancreatic cells cannot be grown in culture, but immortalised *in vitro* insulinoma or tumour exocrine cell lines are available and pancreatic progenitors can be obtained from mouse ESCs and human pluripotent human cells (ESC and iPSC). These alternative models have been used to characterise regulatory intrinsic pancreatic factors. However, pancreas organ development relies on the continuous crosstalk between multiple cell types. These interactions cannot be adequately recreated in culture. Moreover, differentiated ES cultures have a limited lifespan of approximately 3-4 weeks. Therefore, our studies start *in vitro* using cells, but then they require mouse models to assess the complete formation of the pancreas *in vivo*. Likewise, pancreatic beta-cell function and blood glucose homeostasis involve complex cross-talks among different tissues within the body, including the pancreas, liver, muscle and fat. Thus, these processes can only be studied in animals, making *in vitro*

studies insufficient.

Which non-animal alternatives did you consider for use in this project?

Immortalised insulinoma or tumour exocrine cell lines are available and pancreatic progenitors and immature beta-cells can be obtained from mouse ESCs and human pluripotent human cells (ESC and iPSC). Histological sections of human pancreatic tissues are available for analysis. Data sets of gene expression and genomics as well as diabetes (T2D) genome-wide association studies (GWAS) are available; it is also possible to model using computational biology cell behaviour and developmental cell lineage decisions.

Why were they not suitable?

Even though large datasets analysis and mathematical modeling are very effective at hypothesis generation, biological validation requires mechanistic experiments that can only be carried out in cell culture or in mice. Cell culture cannot mimic the full complexity and extended time course of the interactions between different cell types that normally control pancreatic tissue development, homeostasis, or disease. Moreover, the properties of cells change in *in vitro* culture, for example they can lose their differentiated traits, and, therefore, they are not an adequate model for studying the biology of the pancreas or other endodermal-related organs, like the liver.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

My previous licences used a similar number of total mice. The estimated total number of mice in the new licence is based on the assumption that the number of animal experiments performed by my lab will remain the same as in the previous 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In all our studies we aim at reducing animal numbers to a minimum by using the NC3R's Experimental Design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) web application and the ARRIVE guidelines. I obtained advice on statistical analysis from experts, who published books on the design of animal experiments, and gave classes during the PPL holder training course. Also, during the course of the project, to improve the design of animal experiments I can consult our in-house statisticians for advice, as I did in the past in my previous Institute.
Prior to performing an animal experiment, we perform statistical analysis to ensure that we use the minimum number of mice that will be informative. The unit of analysis will be one embryo or one mouse, according to the type of study. Significance will be determined by the unpaired t-test for biological effects with an assumed normal distribution or two-way ANOVA, and $P < 0.05$ will be considered a significant difference. We will usually analyse a minimum number of 8-15 embryos per time-point or 812 adult mice per experimental condition, based on prior experience that this is sufficient to obtain statistically robust data. However, the precise group sizes for individual experiments will depend on the specific question to be addressed and what is being measured. For example, for timed-mating experiments, we can follow the pregnancy by weighting the plugged females before

sacrificing them and avoid false pregnancy. If the animals have not gained weight, they are monitored for the duration of minimum a month after the plug and afterwards they can be used again for future timed-mating experiments. This is a simple and effective method for reducing animal use.
In my laboratory, when planning experiments involving a new application (for example AAV injection of a transgene), we begin with a pilot experiment before setting up the full experiment. Our use of viral vectors (AAV) to introduce genetic mutation helps us to reduce overall animal numbers as the animals carrying genetic mutations of interest would otherwise have to be produced using complex breeding schemes and intercrosses. In some experiments where AAVs are not suitable, a large number of animals have to be bred to obtain sufficient animals of the correct genotype. For example, we may need to activate a fluorescent reporter gene (mouse 1) by crossing it with a mouse expressing Cre
recombinase in a particular cell type (mouse 2) and a mouse in which we genetically knock-out a gene (mouse 3) or we might need to generate a compound mutant mouse (knockout for two genes). Our strategy in these cases is to use littermates that lack one or more of the transgenes as controls in the experiments. Littermate controls provide an extra level of control to our experiment and also reduce the need to set up extra breedings, lowering the overall animal numbers.
Comparison between groups or experimental situations are controlled by **(i)** using inbred animals, minimising genetic variability, and **(ii)** by analysing samples from each group within one evaluation session or test method. For example, sections and immunostainings of animals collected during multiple time points will be carried out in a single instance. This will ensure reproducibility and consistency of data across different groups. Objective analysis by digital imaging is ensured by blinding for group numbers and genotypes. Also, the independent assessment through an image analyst further ensures an unbiased analysis.
Biological variability in animal cohorts is minimised by housing all animals in a controlled environment that is monitored for light cycle, sound, temperature, and humidity. Littermate controls will be used wherever possible. Researchers are blind to the genotype of the animals being injected to prevent any bias.
Finally, we encourage labs that have an interest in other tissues to obtain necropsy samples from our mice. This allows other researchers to obtain preliminary data without having to generate their own mice. In order to reduce the number of mice, we keep stocks of frozen sperm and embryos so that if a line of mice is not required for several months we avoid unnecessary breeding.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Whenever possible lines are maintained as homozygotes to increase the frequency of mice being born with the required genotype. This allows efficient breeding and is a measure to minimise the number of mice we use. To reduce the effects of normal biological variation, mice will be maintained on the same genetic background (C57/Bl6). We perform pilot studies prior to embarking on a new set of experiments. Computer simulation can be particularly informative on initial planning of experiments and for planning more targeted experiments. We encourage sharing of tissues, so that spare tissue from mice can be analysed to obviate the need for killing animals for tissue collection. We have regular meetings of mouse users within our Centre to ensure that lines of animals are not being maintained unnecessarily and can be shared with other users.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and

methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse is ideal for our work as they are a well-established system to mimic human physiology and diseases. In particular, mice show similar glucose homeostatic mechanisms to humans, so data obtained are valuable for translation studies in humans. They have a relatively short gestation period and life span, their biological systems are well characterised in the literature, and their popularity in other research in the field will enable our data to be directly relevant and comparable for other researchers. Lower vertebrates, such as amphibians or fish, are excellent models to study developmental processes, but are relatively limited for understanding glucose metabolism and pancreatic diseases.
Mice are readily maintained in the laboratory and easily subjected to genetic modification. Years of research have refined conditions to provide an optimally enriched laboratory mouse environment and procedures to minimise stress and discomfort. Our mice are bred to a genetically uniform background allowing us to investigate the effects of specific genetic manipulation. Whenever possible, we use genetic mouse models to specifically target the genes of interest in the cells of interest (Cre-transgenic lines), reducing unwanted systemic effects, and under temporal control (TAM- or Dox-inducible transgenes), controlling the time window when mice display a phenotype. When a new sampling procedure is involved, training on dead animals is first carried out. When a new labelling agent or AAV is being tested, an initial experiment is carried out with a small number of mice using the conditions predicted from the literature to be most effective, to ensure that the injected substance does not result in

generalised adverse effects. The data from the first experiment then informs the design of subsequent experiments, which may involve larger numbers of mice. In the case of administration of agents, the table of maximum dosing volumes and frequencies that is included in each protocol will be used. In studying glucose homeostatic mechanisms, the animals suffering during the glucose or insulin tolerance tests is minimised by using blood glucose meters that require below 1 ul of blood, which can be achieved by pin prick to the tail, like a pin prick in humans. Also, when possible we will reduce the period of fasting prior to glucose tolerance tests.

Why can't you use animals that are less sentient?

The goal of our research is to understand mechanisms regulating pancreatic organ formation but also homeostasis and disease in adult pancreatic tissue, and therefore confining our analysis to embryos is not appropriate. Mice are the least sentient mammals that are suitable for the proposed experiments. Moreover, the mouse is the genetic model system, anatomically and phylogenetically closer to humans compared to amphibians or fish, being therefore highly suited to study pancreatic functions, diabetes and to advance therapies to cure diabetes. No surgical procedure is included in our study. Mild animal suffering in this study (*e.g.* due to injection of agents or blood collection) will be minimised by aseptic procedures, but terminal anaesthesia is not suitable to the type of procedures.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Throughout the period of the licence we will seek, and implement, ways to refine the procedures. All of the procedures included in the licence were in use in my previous licences in Germany. The most likely adverse events are not due to the procedures themselves, but might occur to genetic mutations or transgene expression in the mice or development of sporadic tumours. Strong communication is important between researchers and BSU staff to avoid animals going beyond their end points and to ensure that everyone involved is aware of what additional care is needed. In particular, the weights and overall condition of the mice is monitored at least twice a week and once any deterioration is seen the mice are monitored and weighed daily. During glucose metabolism studies (GTT or ITT) the mice being treated will be monitored very closely, in particular in the early stages, to ensure that any unexpected issues are caught rapidly. Also, the BSU staff will be informed and the cages display a notice.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow regular updates on the Home Office website guidance on animal testing and research. We will read the specialist literature, such as the UFAW (Universities Federation for Animal Welfare) journal. We will attend events organised by the local AWERB. We will also check the NC3Rs website on a regular basis (https://www.nc3rs.org.uk/).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Members of my laboratory and I are in contact and receive regular updates from staff members of the BSU in a variety of formats. Home Office recommendations are forwarded to us and we receive invitations to workshops and update meetings. The Home Office Inspector is always very helpful in responding to queries face-to-face or via e-mail. Our NC3Rs Regional Programme Manager, who keep us regularly informed about the latest updates from NC3Rs.

Home Office

NON-TECHNICAL SUMMARY

15.Bone marrow adipose tissue and adiponectin as regulators of caloric restriction and ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Bone marrow adipocytes, Caloric restriction, Ageing, Metabolism, Sex differences

Animal types	Life stages
Mice	adult, juvenile, aged, embryo, neonate, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to identify the mechanisms underlying the health benefits of caloric restriction (reduced food intake without malnutrition) and the development of ageing-related diseases, including how these phenomena differ between males and females. Our focus is on the roles of bone marrow adipocytes (BMAds), the fat-storing cells in our bone marrow; and adiponectin, a BMAd-derived hormone that may have beneficial effects on immunological function and cardiovascular and metabolic health.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Scientists first noticed the existence of BMAds over a century ago. Having fat in our bones might strike you as unusual, but it is not: BMAds make up to 70% of bone marrow volume in healthy adults, suggesting a role for them in normal human physiology. BMAds further accumulate with ageing and in conditions of altered skeletal or metabolic health. For example, BMAd numbers increase during osteoporosis, suggesting that they might promote bone fragility. Increases in BMAds also occur with ageing and in obesity, suggesting that they might influence the progression of age- and/or obesity-related diseases. Perhaps most bizarrely, BMAd formation increases during states of caloric restriction (CR), a health-promoting dietary intervention in which food intake is decreased but without causing malnutrition. This property of BMAds is in stark contrast to adipose tissue elsewhere in the body, called white adipose tissue (WAT), which is broken down during CR to supply energy. CR has numerous health benefits, including decreased risk of cancer, diabetes and cardiovascular disease. These observations suggest that, like WAT adipocytes, BMAds might directly impact metabolic health. However, unlike WAT, little is known about the biological functions of BMAds.

Previous research from my lab has begun to elucidate the functions of BMAds. In particular, CR is associated with increased circulating levels of adiponectin, a hormone implicated with beneficial cardiovascular and metabolic effects. I discovered that BMAds are a key source of adiponectin during CR. This suggests that adiponectin and BMAds might contribute to the health benefits of CR. It also raises the possibility that BMAds contribute to increases in adiponectin in other contexts. For example, ageing is also associated with BMAd accumulation and increased circulating adiponectin; however, how BMAds and adiponectin influence the pathophysiological effects of ageing remains poorly understood. Finally, my lab's research has identified striking sex differences in the effects of CR and ageing, but the basis and extent of these remain to be firmly established.

The work to be pursued under this project license is important because it will establish the role of BMAds and adiponectin in the sex-specific effects of CR and ageing. These include effects on immunological function; the formation and maintenance of bone tissue; and our metabolic and cardiovascular health. This knowledge is relevant not only to fundamental understanding of key biological processes, but also to diseases that are placing a major burden on the health of our society. Pursuing this work may therefore help to identify new approaches for the prevention and treatment of these diseases.

What outputs do you think you will see at the end of this project?

This research will generate new knowledge of the fundamental biology of bone marrow adipose tissue (BMAT) and adiponectin, including their metabolic and endocrine functions; whether they contribute to the cardio-metabolic, skeletal and/or and immunological effects of caloric restriction and ageing; and the extent and basis

of sex differences in the effects of ageing and caloric restriction. These advances may help to design improved strategies to benefit human health, including the diagnosis, prevention and treatment of diverse diseases.

This new knowledge will be communicated in scientific publications and, where relevant, in the general media. In the longer-term it may also yield products relevant to the use of BMAT or adiponectin as therapeutic targets and/or biomarkers for treatment efficacy and/or disease risk.

Who or what will benefit from these outputs, and how?

A primary benefit of this new knowledge will be for scientists with interests in metabolic regulation, endocrinology, caloric restriction, gerontology, cardiovascular biology, adipose tissue and bone development, bone remodelling, immunology, biomedical imaging, machine learning and population-level studies.

A secondary benefit is the translational potential of this work. To complement our studies in animal models, we are pursuing three related lines of clinical research. One study involves the isolation and characterisation of BMAT from human patients, including those undergoing surgery for hip replacement, knee replacement, or ankle fractures. A second group of studies involves the use PET-CT and PET-MRI imaging to non-invasively assess the metabolic functions of BMAT in humans. Finally, we are using machine learning to identify BMAT in up to 100,000 participants from the UK Biobank study, which will allow genomic approaches to dissect BMAT formation and function. We are also utilising such population-level approaches to assess the functions of adiponectin, and sex differences in health and disease. The combination of this clinical research with the mechanistic insights generated through our animal studies may, in the longer-term, facilitate the development of new drugs that target BMAT and/or adiponectin to benefit immune function, cardio-metabolic and skeletal health.

As one example, our research will reveal if BMAT contributes to glucose clearance in conditions of CR and whether this is altered in states of obesity, diabetes and ageing. If so, then BMAT might represent a therapeutic target against diabetes. Similarly, our infection studies in both wild-type mice and mice genetically modified to express no adiponectin will reveal new knowledge about this widely studied hormone, including how it influences the impact of age, sex, and obesity/diabetes on immune function. This knowledge is directly relevant to our understanding of risk factors for COVID-19 and other infectious diseases.

Improved knowledge of BMAT function could also benefit current diagnostic practices. For example, BMAT is emerging as an important clinical indicator for increased fracture risk, and therefore could be used as a biomarker to identify high-risk individuals. Our research will reveal if BMAT also exerts systemic metabolic effects, in which case BMAT might be associated with altered cardiometabolic health. Such knowledge would better inform clinicians and public health policymakers in using BMAT as a clinical biomarker.

Finally, our research may establish new methods for BMAT analysis that will benefit research and potentially allow development of new clinical approaches.

In summary, the research described in this license will reveal new fundamental knowledge of BMAT and adiponectin, including their therapeutic and pathophysiological relevance, and will develop new methods with potential to benefit basic research and clinical practice. Such knowledge might benefit diverse clinical settings and therefore has widespread translational potential.

How will you look to maximise the outputs of this work?

We will maximise our research outputs in several ways. Firstly, all of our data, protocols and study designs, including the results of unsuccessful approaches, will be made publicly available. This will include publishing in open-access journals and depositing information in open-access repositories such as protocols.io and the NCBI GEO platform. Secondly, we collaborate widely with researchers from across the diverse fields relevant to our research, including metabolism and endocrinology, cardiology, bone biology, immunology, biomedical imaging, machine learning, and genomics. These collaborations allow us to maximise the insights gained from our research and to share methods and resources, thereby enhancing research impact. Finally, to facilitate translation and innovation we have established close working relationships with our university's innovation team, who provide expertise in facilitating the commercialisation and translation of research.

Species and numbers of animals expected to be used

- Mice: 4000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use various strains of common laboratory mice, because these are well-characterised, reliable models of human health and disease.
These species also allow genetic modifications and other interventions that are extremely useful for identifying the basis for biological processes and disease progression.

We typically study sexually mature adult animals (0-12 months of age) and for some studies will study aged animals (12-24 months of age); the latter are used to investigate ageing-associated changes linked to increased risk of chronic diseases such as diabetes, heart disease, and osteoporosis.

Typically, what will be done to an animal used in your project?

Our typical interventions include caloric restriction (CR) and ageing. Substances, such as hormones or drugs, may be administered to animals by injection or using devices implanted subcutaneously.

Gonadectomy (removal of the ovaries or testes) may be done to test the basis for any sex differences in cardiometabolic, skeletal or immunological functions.

Metabolic function may be tested using imaging studies; tolerance tests and/or injectable tracers to assess glucose and fat metabolism; and other methods to determine insulin responsiveness.
Cardiovascular function may be tested by measuring blood pressure; using ultrasound to analyse heart function; and through surgeries (done under terminal anaesthesia) that mimic heart attacks. Skeletal function is tested using cell labelling to measure rates of bone formation, and by analysing the concentrations of factors in the blood that indicate bone formation and turnover. Immunological functions are tested by sampling blood for analysis of white blood cells.

What are the expected impacts and/or adverse effects for the animals during your project?

CR causes decreased body weight and minor bone loss, but not to a pathological extent. Ageing is associated with increased incidence of spontaneous tumours and can feature other comorbidities. Thus, aged animals are monitored weekly for clinical signs of illness and removed from studies before undue suffering occurs.

Very little mortality is associated with device implantation, while mortality rates from gonadectomy are low (<1%). A minority of animals (<5%) will die during surgeries that mimic heart attacks; however, they will be under general anaesthesia, preventing any pain or suffering. Tests of metabolic, cardiovascular or skeletal function generally do not cause adverse effects. In all studies, we carefully monitor animals to ensure that moderate severity levels are not exceeded. Animals are humanely euthanised at the end of each study, once we have gathered the necessary information.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately half of the mice will be used for breeding purposes and are expected to experience mild severity. The remaining mice will be used to investigate experimental objectives and are expected to experience moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Caloric restriction induces interconnected physiological effects on diverse tissues, including the brain (e.g. appetite), the skeleton (e.g. BMAT expansion), adipose tissue (e.g. decreased lipid storage), and muscle (e.g. increased fat oxidation). Ageing also causes numerous systemic metabolic effects, while sex differences typically relate to complex multi-organ actions of sex hormones. Studying such effects is not possible *in vitro*; hence, there is no way to replace the insights gleaned through animal studies.

In addition to studies in live animals, we extensively analyse animal tissues taken post-mortem and human tissues from clinical studies. These approaches support and complement our investigations in live animals.

Which non-animal alternatives did you consider for use in this project?

In vitro cell culture using cell lines such as 3T3-L1 preadipocytes and ST2 mesenchymal stromal cells can serve as useful models to investigate the formation and function of cells involved in fat storage (adipocytes) and bone formation (osteoblasts). Therefore, we considered if these non-animal models could be useful to address our

research aims, including how CR and/or ageing regulate fat and bone formation and the function of osteoblasts and bone marrow adipocytes.

Why were they not suitable?

Using cell culture systems, it is not possible to replicate the diverse pathophysiological effects of CR, ageing, or sex differences. Moreover, we and others have found that available cultured cell lines do not faithfully recapitulate the properties of adipocytes and osteoblasts in vivo. Therefore, these alternatives are not suitable for robustly and reliably addressing the aims of this project.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Over this 5-year project we expect to use up to 4000 mice, 60% of which will be for breeding programmes. We will use as few mice as possible to answer our questions, aiming to maximise the amount of information obtained per animal to help realise the potential benefits of our research.

The numbers of mice required for breeding genetically altered strains have been estimated based on refined breeding strategies in which we have extensive experience. The numbers required for our experimental protocols are estimated based on both the numbers of experimental groups/variables within each study (e.g. male vs female, control vs experimental diet, young vs old, control vs experimental substance/agent, etc...), and the number of animals needed within each group. The latter has been estimated based on the results of previous studies (from our lab and/or others) that have used similar model systems to investigate the effects of variables of interest. The effect sizes from these previous studies have been used to estimate the minimum number of animals needed to draw robust and reliable conclusions from our experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The estimates above incorporate power calculations using G*Power software and, where necessary, consultation with statisticians. To further reduce the overall number of animals required we:

- Use inbred mice to reduce inter-animal variability.
- Where possible, increase statistical power by using a multi-factorial design that incorporates several different experimental variables. Such variables may include sex, diet, genetic modification, surgical intervention, control vs experimental substance/agent (etc...). Including multiple variables within a single study provides more power to detect effects of each individual variable and the interactions between them, thereby decreasing the numbers required per group. In addition, such multi-factorial designs prevent the need for separate studies that each assess the effects of a single variable, and which would each require their own dedicated control groups. By doing so, multi-factorial designs reduce the numbers of separate control groups and thereby reduce overall animal numbers.

- Maximise the use of non-invasive techniques (e.g. non-invasive imaging, tail cuff blood pressure measurement) and other methods that allow repeated measurements within a single animal. This increases statistical power and reduces the total number of animals required.

- Use other advanced techniques that allow reliable results to be obtained using fewer animals (for example, the use of dynamic PET/CT rather than static PET/CT analysis).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For breeding of experimental animal lines we use the most refined and efficient breeding strategies. We always take into account the ages at which males and females have the greatest fecundity. For genetically altered strains that require more-complex breeding strategies we have developed breeding strategies that maximise efficiency while minimising potential adverse effects.

For experimental protocols we perform pilot studies to identify the parameters that minimise adverse effects while producing the most robust, reproducible experimental readouts. This enhances the effect size of variables of interest, allowing fewer animals to be used to obtain reliable results.

We embrace open science principles and collaborate extensively within our University and beyond. Regular meetings within our research group and with other researchers ensure that maximal data is obtained from animal studies. We make datasets and methods publicly available (i.e. via public repositories) and, wherever possible, we encourage other scientists to analyse some of our postmortem animal tissues. These open, collaborative approaches provide valuable insights into other fields (e.g. cancer, haematology, evolution/ecology, reproductive health) and reduce the need for additional animals to be used by others.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use mice on a particular in-bred genetic background called ‘C57BL/6’ because this has been used extensively to refine the experimental procedures of interest, including surgeries, skeletal and metabolic analyses. The prior optimisation of these procedures in C57BL/6 mice makes these mice the ideal model for pursuing our research while causing the minimum stress and suffering to the animals. In addition, the C57BL/6 background offers a range of genetic modifications (e.g. lack of the hormone adiponectin; effects on sex hormones) that are the most refined models possible for addressing our research objectives.

For caloric restriction (CR), animals are fed a micronutrient-enriched diet to avoid malnutrition. Previously, CR has required mice to be singly housed; however, we are now exploring the use of cage dividers during food administration, to allow mice to undergo CR whilst being group housed. Ageing studies are done in

animals below two years of age to ensure a low incidence of age-related comorbidity while still allowing development of experimentally and clinically relevant age-related changes.

We progressively develop and refine our methods. For example, our metabolic analyses begin with exploratory methods (e.g. insulin tolerance tests) to assess if experimental interventions have major effects on broad outcomes (e.g. “insulin sensitivity”). Only in the presence of a clear effect are more in-depth methods used, such as using infusions of labelled nutrients (e.g. glucose) and tracking their fate in a living animal. Such in-depth methods allow us to better determine the mechanisms underlying major effects. For such methods, the use of any invasive (e.g. surgical) techniques is discussed with colleagues performing similar work locally and across the country. We will tailor monitoring systems to each model and apply strict humane endpoints to minimise suffering.

Why can’t you use animals that are less sentient?

Immature life stages cannot be used for two main reasons. Firstly, a key aim of our research is to determine the basis and extent of sex differences in the effects of caloric restriction, obesity and ageing.

Such sex differences typically depend on animals first reaching sexual maturity. Secondly, our research into ageing necessarily requires the use of aged animals.

We are unable to use less sentient species because we seek to reveal new knowledge of diverse pathophysiological processes relevant to human health. These processes include metabolic and cardiac function, bone biology and immunological responses, and whether these differ between males and females. The need to assess bone biology necessarily precludes the use of invertebrates. The need to assess sex differences would greatly complicate the use of lower invertebrates, e.g. zebrafish, because their sexual characteristics (e.g. hormones, method of sex determination) are very different to those of mammals. Finally, the pathophysiological processes that we study are often influenced by interconnected whole-body effects, e.g. mediated by hormones and interactions between organs and tissues. Studies of mammalian species are therefore required if we are to gain insights that are closely relevant to human health.

We do use adult animals that are terminally anaesthetised for some procedures, including surgical models of heart attacks, and in tracer studies that allow us to analyse carbohydrate and fat metabolism in living animals. This minimises the suffering experienced by these animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Throughout our studies we strive to minimise suffering. We closely monitor animals for adverse effects (e.g. comorbidities associated with ageing, CR, surgeries, or other experimental interventions). In some experiments the combination of procedures and conditions may exacerbate adverse effects on animal welfare. For example, gonadectomised mice might display an enhanced adverse responses during CR, such as weight loss or hypoglycaemia. In such cases we increase the frequency of animal monitoring and, should adverse effects develop beyond defined limits, steps are taken to address these. These monitoring and treatment strategies minimise welfare costs for the animals.

For other procedures, associated stresses can be minimised by training the animal. For example, placing mice in restraint tubes is required for measurement of body composition or blood pressure, but these restraints can increase stress to the mice. In such cases the animals are habituated to these conditions, which minimises the stress response. For surgical procedures, appropriate anaesthesia, analgesia, postoperative care and aseptic techniques will be used. Surgeons are thoroughly trained and rigorously assessed prior to performing surgery on a live animal. Substances will be administered at non-toxic doses and through the least invasive routes, e.g. in

food/water. If injection is necessary, needles will be used only once. For some metabolic analyses we use new low-stress procedures that minimise suffering while maximising the amount of information obtained per animal, e.g. non-invasive imaging for fat mass determination, or indirect calorimetry to assess energy homeostasis. Finally, we will continue to implement refinements in animal handling, such as use of non-aversive mouse handling methods.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will use the refinement resources on the NC3Rs website to identify guidance for continued refinement of our experiments, including animal handling (https://www.nc3rs.org.uk/handling-and-restraint), use of anaesthesia (https://www.nc3rs.org.uk/anaesthesia), and implementation of humane endpoints (https://www.nc3rs.org.uk/humane-endpoints). Other publications will be consulted for guidance on more-specific procedures, e.g. experiments to assess metabolic function (https://pubmed.ncbi.nlm.nih.gov/20713647/).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I receive the monthly NC3Rs newsletter. In addition to this, my university organises a frequent seminar series focussed on the 3Rs, with seminars typically held fortnightly or monthly. My lab members and I will frequently attend these seminars and consult with animal care staff, including Named Veterinary Surgeons and Named Animal Care and Welfare Officers, to identify strategies to implement any advances in our project.

Home Office

NON-TECHNICAL SUMMARY

16. Brain Mechanisms of Cognition & Behaviour

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Brain, Behaviour, Cognition, Rat model

Animal types	Life stages
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We will study brain mechanisms of cognitive functions (such as memory, attention and behavioural flexibility) and other behavioural functions (including sensorimotor functions, such as locomotor activity, and motivational functions) and how aberrant brain function causes clinically relevant impairments in such cognitive and behavioural functions. We will focus on the 'hippocampus', a brain region underneath our temples, the 'prefrontal cortex', a region at the front of the brain, and 'subcortical' regions, regions that lie deep in the brain, and on the role that inhibitory control of these regions by the brain chemical GABA plays in the function of these brain

regions.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Generally, there is a strong public interest in understanding brain-behaviour relations in health and in disease. Behavioural and cognitive impairments due to brain disorders are a major cause of functional disability and economic burden in the UK and worldwide, but development of efficient treatments has stalled, largely due to an insufficient mechanistic understanding of brain-behaviour relations. This project will address aspects of these public and clinical needs.

More specifically, aberrant function of the brain regions studied in this project has been implicated in important psychological problems, including in age-related loss of cognitive functions, such as memory and attention, in schizophrenia and in Tourette's (a disorder characterised by 'tics' - involuntary movements and sounds that the patient cannot control). Moreover, impairments in the inhibitory control of neuron firing in these brain regions by the brain chemical GABA has emerged as a key feature of these disorders. Therefore, it is important to understand better how inhibitory control of neuron firing by GABA contributes to how the brain produces behavioural and cognitive processes.

What outputs do you think you will see at the end of this project?

The research project will produce new information to improve our understanding of the brain mechanisms of normal cognition and behaviour, as well as of the pathophysiological mechanisms relevant to neuropsychiatric diseases, including age-related cognitive decline, schizophrenia, and Tourette's syndrome.

To disseminate our findings to other academic, clinical and industry researchers across the world who focus on brain-behaviour relations in health and disease, we will publish our findings in high-quality peer-reviewed research papers in high impact academic journals. In addition, we will disseminate our findings at national and international scientific meetings and at seminars.

Our findings will also inform our teaching of undergraduate and postgraduate students and will be communicated to the general public as appropriate (via our webpages and press releases, Brain Awareness Week events, Summer Schools for school students, Pint of Science events, articles in popular science journals, etc.).

Who or what will benefit from these outputs, and how?

Outcomes of this research will immediately improve understanding of the brain mechanisms of normal cognition and behaviour, as well as of the pathophysiological mechanisms relevant to neuropsychiatric diseases, including age-related cognitive decline, schizophrenia, and Tourette's syndrome. This addresses the strong public interest in understanding brain-behaviour relations in health and disease.

Cognitive and behavioural dysfunctions due to brain disorders are a leading cause for functional disability and a major socio-economic burden in the UK and worldwide, but development of efficient treatments has stalled. A mechanistic understanding of brain-behaviour relations and of the pathophysiology of these disorders is likely the best hope for better treatments in the long-term. Some of our in vivo research is in collaboration with drug discovery industry partners who are involved in applied research with the aim to discover new medicines that improve cognitive impairments. These industry partners and their drug discovery research programme will immediately benefit from the outcomes of our research, which will help them in their prioritisation of promising neural mechanisms that could be targeted by new pharmacological treatments.

How will you look to maximise the outputs of this work?

We aim to publish comprehensive, high-quality peer-reviewed papers, which we make available for open access.
Throughout the project, we will present preliminary findings in form of poster presentations and talks at international conferences and through other invited talks. In addition, once papers are completed for submission to peer-reviewed journals, we will place them on a pre-print server (bioRxiv). This will ensure that our findings are disseminated as swiftly as possible. It has the added benefit that the progress of our project can benefit from early feedback by peers.
We also aim to broaden the benefits of our research by including our research findings in our teaching to undegraduate and postgraduate students and by disseminating it to the general public via our webpages and press releases, Brain Awareness Week events, Summer Schools for school students, Pint of Science events, articles in popular science journals, etc.

To maximise the impact of our research on the discovery of new pharmacological treatments, we collaborate with colleagues from the neuroscience drug discovery industry.
We also closely integrate our in vivo research in rats with non-invasive studies in human participants, including patients, that uses similar behavioural and non-invasive imaging methods. This will help to maximise the translation of our findings to humans.

Species and numbers of animals expected to be used

- Rats: 1,000 rats

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use young adult rats, because their brains are similarly organised as in humans, and a wide range of behavioural tests is available to study the cognitive and behavioural functions of interests. In addition, the welfare demands of rats can be well satisfied in captivity.
The brains of rats are large enough to manipulate and analyse specific brain regions with high selectivity, and behavioural tests are well-established in this species, because it has been used for research for a long time.

Typically, what will be done to an animal used in your project?

To examine the brain mechanisms of cognitive and behavioural processes, rats will undergo behavioural testing while specific neural mechanisms are being manipulated. These manipulations can be brain site-specific or systemic. Brain site-specfic manipulations require that the rats undergo surgery under full anaesthesia, so that we can manipulate a specific brain region or implant cannulae into the brain region of interest, so we can later manipulate the brain region by infusion of small quantities of neuroactive compounds, which modulate neural activity. Systemic manipulation of neural activity will involve the systemic administration of a neuroactive compound. The behavioural testing procedures often rely on the rats' spontaneous behaviour. For example, we use the rats' spontaneous exploration of an environment or object to measure their lococomotor activity or their 'interest' in an object. The latter allows us to measure the rats' memory of this object, as rats are more

'interested' in novel objects (i.e., the more they explore the object during a test, the less they remember it from a previous training session). In other procedures, we require rats to attend to and remember certain stimuli and procedures in order to receive food reward or to find a hidden platform that offers escape from a pool of water (note that rats are very good swimmers!).
Complementing our behavioural studies of the effects of specific manipulations of brain function, we also use direct electrophysiological recordings of neural activity, by insertion of electrodes into specific brain regions, or the non-invasive measurement of brain function by magnetic resonance imaging methods (similar to those that are used in clinical studies to measure brain function in human participants). These procedures will be performed under anaesthesia and tell us how a brain manipulation specifically affects the activity of neurons and the pattern of such activity within different brain regions.
At the end of the studies, all rats will be humanely killed.

What are the expected impacts and/or adverse effects for the animals during your project?

The welfare demands of rats can be well satisfied in captivity. The welfare of our animals is important for the success of our studies, which would be confounded by undue discomfort and stress of the animals.
Our experiments require surgical procedures to manipulate selected brain sites and monitor their function. These procedures are performed under full anaesthesia, complemented by appropriate analgesia, and typically do not cause lasting discomfort. In fact, following a few days of recovery, rats are ready to take part in our behavioural tests that require attention and inquisitiveness.
Some of the behavioural procedures we use to assess cognitive functions require mild to moderate aversive motivation, by requiring escape from water by swimming to an easily accessible platform. Others rely on the motivation to ‘work’ for food and require that the animals are maintained with restricted access to food. The discomfort caused by these procedures is mild to moderate, and probably less severe than that experienced by wild rodents due to predators and other natural stressors. By examining how manipulations of neural activity in specific brain sites change the animals’ performance measures on our behavioural tests, we can find out how these brain sites contribute to the behaviours under study.
The impact of the manipulations of brain activity would typically result in specific impairments in selected cognitive and behavioural processes. These impairments would often not be visible to the naked eye, but their detection and measurement would require dedicated and sensitive behavioural assays. As the manipulations of brain activity are temporary, the behavioural and cognitive impairments are temporary, too.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

It is expected that virtually all rats used in this project will expect temporary moderate severity, due to the surgical procedures, the manipulations of brain function, the behavioural testing procedures or a combination thereof.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The detailed mechanistic understanding of causal brain-behaviour relations requires the combination of behavioural testing with invasive methods to manipulate and analyse function of brain sites with high specificity. For ethical reasons, this is impossible in human participants, and animal experiments are necessary.
Therefore, no alternatives can fully replace the in vivo animal experiments to achieve our aim and objectives.

Which non-animal alternatives did you consider for use in this project?

Although no alternatives are available to fully replace our animal experiments, our experiments in rats are informed by and closely integrated with complementary non-animal approaches. Non-invasive brain imaging studies in humans reveal important brain-behaviour correlations, although they cannot reveal the causal roles of specific brain processes in a behavioural phenomenon. We collaborate closely with colleagues studying related topics in human participants, using non-invasive brain imaging. For example, based on our behavioural tests in rats, we have developed similar behavioural tests for human participants. Combining these with non-invasive imaging in human participants, we can reveal neural correlates of the cognitive and behavioural processes of interest, complementing our invasive studies in rats. Even though these studies cannot replace the proposed animal experiments, a close integration of studies in humans and in animal models will help to maximize the benefit of animal experiments. Moreover, our clinically motivated animal experiments are strongly informed and, in some instances, are directly based on clinical non-invasive imaging studies in humans. For example, our rat studies involving non-invasive brain imaging methods in rats are aimed at revealing the causes for changes in brain imaging measures that have been observed in patients of neuropsychiatric disorders, including schizophrenia, age-related cognitive decline and Tourette's Syndrome.
In vitro studies in brain slices can reveal important basic neurophysiological processes, but in vitro results are difficult to link directly to behavioural studies. Moreover, neuronal interactions within extended structures, such as the hippocampus, and between brain structures are difficult, if not impossible, to study using brain slices in vitro, as the brain "slicing" destroys the relevant neuronal connections.
Using in silico computational methods to model the neurochemical and neural circuit mechanisms of cognition is possible to some extent, but these models depend on the type of data generated by experiments as proposed in this project. We have begun collaborative work with neurocomputational scientists to integrate our data into neurocomputational models. Even though such models cannot replace the proposed animal experiments, such models help to maximize the conceptual benefit of our experiments and they will enable us to devise more accurate and specific hypothesis.

Why were they not suitable?

Please see section above for why potential non-animal alternatives are not suitable to fully replace our animal experiments.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The animal numbers required for individual experiments have been estimated to be as small as possible, while still enabling us to reveal reliably the effects of interest.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We plan each experiment carefully (using the NC3R's Experimental Design Assistant, if appropriate) and use state-of-the-art methods to minimise variability and to obtain robust, sensitive and reliable measures with the minimal number of animals. Experimental procedures will be standardised as far as possible to avoid unnecessary variance.
Where possible, within-subjects designs will be used, so that each animal serves as its own control in order to reduce variance and the number of animals. However, some experiments require between subjects factors, e.g. when the measure under study changes due to repeated testing. To increase the probability of revealing real effects of our manipulations, rather than spurious effects caused by confounding variables, the influence of confounding extraneous factors will be minimised by random allocation, counterbalancing and/or matching, and blinding as appropriate.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

As indicated under Replacement, our animal experiments are closely integrated with non-invasive brain imaging studies in human participants and with neurocomputational modelling work.
Our in vivo experiments are complemented by ex vivo measurements, e.g. of how the manipulations we use affect certain neural markers.
If we run larger studies, which are completed using several batches of rats, we will stop the study for 'futility' if the results obtained with the first batches of rats indicate that we will not be able to detect the predicted effect (this will be done using statistically sound criteria).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Our experiments are conducted in rats, because their brains are similarly organised as in humans and a wide range of behavioural tests is available to study the cognitive functions of interests. In addition, the welfare demands of rats can be well satisfied in captivity.
The brains of rats are large enough to manipulate and analyse specific brain regions with high selectivity and behavioural tests are well-established in this species, because it has been used for research for a long time.
Our main experimental tool to manipulate brain function is the temporary pharmacological manipulation of specific brain sites in awake rats by local microinfusion of neuroactive drugs via pre-implanted cannulae. A main focus is on manipulating regional inhibitory signalling between neurons by infusing drugs that block the actions of the main inhibitory neurotransmitter GABA. The regional infusion of such drugs mimics aspects of regional GABA dysfunction associated with neuropsychiatric disorders, including schizophrenia, age-related cognitive decline and Tourette's. We have long-standing experience with the intra-cerebral micro infusion method and perform this to the highest standards, using fine-gauge cannulae and small infusion volumes to minimise non-specific neuronal damage and mechanical effect of the infusions and to limit the regional spread of the drug. This approach enables us to study specifically the role of GABA-mediated inhibition within specific brain sites. Furthermore, the reversibility of pharmacological manipulation enables us to study brain substrates of behavioural processes with fine temporal resolution (e.g., we can test if a brain region contributes to learning or retrieval of information by selectively switching it off during one of the two stages); reversibility (due to ‘wearing off’ of the drug effect within typically a few hours) also enables within-subjects studies (helping to reduce the required number of animals), which are not possible with permanent lesions. To complement drug microinfusion

studies and examine directly the functions of inhibitory neurons that release GABA, we will use new pharmacogenetic DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology. In addition to the advantages of drug micro infusion approaches indicated above, pharmacogenetic DREADD technology offers us the opportunity to directly manipulate a specific type of neurons in a specific brain region. Using this approach in transgenic rat lines expressing Cre-recombinase in GABAergic marker genes, this approach enables us to study directly the functions of GABA-releasing inhibitory interneurons in specific brain regions. This is important, because regional malfunction of these neurons has been implicated in many neuropsychiatric disorders, including schizophrenia and Tourette's.
Animal welfare is vital for the success of our studies: behavioural tests require the animals' inquisitiveness and free exploration; insertion of infusion cannulae into pre-implanted guides (as necessary for our studies involving pharmacological manipulation of specific brain sites in behaving rats) requires tame and comfortable animals; undue stress would interfere with most of our measures. Surgical procedures are conducted under aseptic conditions and under appropriate general anaesthesia, complemented by appropriate peri-operative analgesia and care. Appropriate recovery from surgery will be allowed before any behavioural testing. We minimise undue stress of the experimental animals by carefully habituating them to the required handling before any procedure. If we require purely physiological measures, without behavioural measures, experiments will be conducted under anaesthesia, thereby minimising suffering. Within our animal facilities, high welfare standards are maintained, and, if appropriate, we seek advice from qualified staff or the vet to minimise any suffering.

Why can't you use animals that are less sentient?

The study of the clinically relevant brain mechanisms and behavioural and cognitive functions of interest require a mammalian species and mature animals.
If we take purely physiological measures of neural activity, without the requirement of behavioural measures or of repeated measures, we perform the experiments under terminal anaesthesia.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All rats used in this project are monitored regularly and carefully, especially following surgical procedures or experimental manipulations that have an increased potential for adverse effects. If the rats show any signs of adverse effects, we will seek the advice of the named persons or vet and remedial action will be taken promptly. Our protocols have clear and well-defined humane endpoints to reduce animal suffering.

Before the start of any procedure, all rats are habituated to the facilities and to the experimenter, by regular handling, so as to minimise any stress and discomfort of rats in relation to handling required for the completion of our procedures.
To minimise pain in relation to surgical procedures, general anaesthesia is complemented by local anaesthesia and appropriate analgesic regimes, based on consultation with the vet.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow guidelines published by NC3R, including the ARRIVE guidelines
(https://www.nc3rs.org.uk/arrive-guidelines
), as well as guidance published by the Laboratory Animal Science Association (LASA)
(https://www.lasa.co.uk/current_publications/).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We receive regular NC3Rs e mails, which keep us updated about advances related to the 3Rs.
We also receive regular updates and latest animal welfare guidance through our Named Competency and Training Officer (NCTO).

Home Office

NON-TECHNICAL SUMMARY

17.BRCA1-associated pathways in cancer development

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

cancer, therapy, cancer development, Hereditary, PARP inhibition

Animal types	Life stages
Mice	adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to investigate distinct BRCA1 pathways and functions in tumour development and identify new treatment strategies for familial breast and ovarian cancer and for other cancers in which the BRCA-pathway is disrupted.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Inheritance of a change in known cancer-preventing gene, BRCA1, PALB2 or BRCA2, can cause breast or ovarian cancer. There have been more than 2500 changes of 'unknown clinical significance' found in patients with family history of cancers in the BRCA1 gene alone. These changes may, or may not, give an increased risk of cancer. A recent test-tube experiment that made many thousands of changes to the BRCA1 gene and predicted that ~4000 changes may cause cancer (Nature 2018). At the moment genetic counsellors are unable to tell patients with a change of unknown clinical significance of their likely risk of disease, and thus cannot advise appropriate management. The reason we cannot tell people of the danger these genetic changes pose is that the precise function of BRCA1 and its associated genes in stopping cancer development is poorly understood.

Most breast cancer patients are treated successfully. In ~20% of patients, their cancer comes back. Breast and ovarian cancers arising as a result of inheritance of defective genes are often sensitive to treatments that targeting the DNA repair deficiency that occurs when the BRCA gene is changed. In particular, these treatments include agents called PARP inhibitors. Cancers that occur without inherited changes in these genes may also nevertheless have defects in the BRCA-DNA repair pathway and so are also candidates for this treatment. These cancers include lymphoma, prostate, endometrial, brain and gastric cancers.

Sadly we know from the investigations of ovarian cancers treated with PARP inhibitors that all eventually continue to grow despite the presence of the drug, so there is a need to identify new treatments. We need to better understand the molecular function of the genes involved in ongoing cancer growth to identify new vulnerabilities that could be targeted to help patients.

We aim to breed animals with different changes in the mouse BRCA1 gene. These changes are designed at specific locations to disrupt particular functions, but not others. By examining the animals we will be able to find out whether these particular functions prevent tumour development. We aim to cross these with other animals with changes in genes that we know alter the way BRCA1 works. For example, one of these is very important only when certain functions of BRCA1 is missing, remarkably its removal allows embryo survival and suppresses cancer development, while in cancers its reduction is associated with therapy resistance.

What outputs do you think you will see at the end of this project?

The principal outputs from this project will be in the form of new information that will be disseminated to the pharmaceutical and academic community via high impact publication and presentations at conferences.

We expect to characterise new models of BRCA1 disruption that may inform mechanisms of cancer development. We believe that this will help to better understand the tumour development process and functions of the BRCA1 gene within it. Given our active collaborations, our results could lead to new treatment strategies and phase I clinical trials for cancer treatments.

Who or what will benefit from these outputs, and how?

The work will add to the knowledge of which BRCA1-mediated pathways are, and are not, relevant to tumour development -accelerating the classification of genetic variants that are currently of unknown clinical risk.

It will accelerate the analysis of which agents are, and are not, effective in treating BRCA1-mediated cancers (output). In the short term, the outputs will accelerate scientific progress towards a greater understanding of BRCA-mediated cancers (output expected during or slightly after the project).
Both the agents used to test effectiveness, and the agents based on targets to be inhibited are available to us and so our evidence may thus accelerate trials and/or identify the patient group that the agents may be effective in (output of the current project expected to be released some 5-20 years after the project depending on the agents used, and whether they are new or already developed.
In the longer term, it is hoped that the work will impact the patients themselves if agents/targets we investigative prove to improve tumour regression (longer-term).

How will you look to maximise the outputs of this work?

To maximise the outputs we are collaborating with different groups internal and external to the establishment that will lend their expertise and knowledge to our work. We will publish our work in peer-reviewed journals and present at conferences to disseminate new knowledge including reporting of unsuccessful approaches. We will also share best practice of new techniques and protocols we have developed and validated with collaborators.

Species and numbers of animals expected to be used

- Mice: 15000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use mice to enable a genetic approach to understand how cancer develops and thus identify novel targets for therapeutic intervention. Mice are the species of choice because there are a large number of widely available genetically modified strains that means that the function of most genes of interest can be studied. Moreover, they are able to grow tumours as engraftments enabling drug testing and in vitro genetic manipulation before implantation. This enables us to mechanistically interrogate tumour growth, and hence identify new ways in which to drug target. We are predominantly using mouse strains that are bearing mutations either found in breast cancer patients or designed to interrogate a particular molecular pathway.
A second reason we have chosen to use mice is that the majority of cancer models we will be using have been extensively characterised previously and develop tumours with a similar aetiology and molecular profile as that observed in human disease. Tumour growth rate is predictable, thus fewer mice are required to be used for each experiment. Some of our studies will involve the generation of novel genetically modified animals, however during this process, all animals will be closely monitored for signs of distress and handled in the appropriate manner.
Also, we need to isolate and immortalise primary cells for further studies. In this case, embryos are isolated from E13.5 enabling the generation and culturing of mouse embryonic fibroblasts. This provides us with a source of genetically altered cells in which we can conduct in vitro experiments in order to better understand molecular mechanisms.

Typically, what will be done to an animal used in your project?

Most of our animals will be set up in breeding pairs enabling the generation of mice with specific mutations. Generation of those strains enables us to investigate tumour development and progression during ageing of animals. Mice used for breeding will undergo a maximum 6 breeding cycles.
Some of the dams will be culled at day 13.5 of pregnancy in order to isolate primary mouse embryonic

fibroblasts.
Some of the dams will have nipple injection of viral particles to induce development of tumour growth in mammary glands.
Animals will have injected or implanted tumour cells or pieces. The tumours could take up to 12 weeks to form. We will closely monitor this process by: 1) caliper measurements, 2) feeling the abdominal area or 3) in some cases live imaging where cells are tagged with fluorescent protein and imaged using IVIS, which involve IP injection of luciferin and general anaesthesia. In some cases we will need to implant tumour material in immune compromised animals to allow tumour growth.
To determine the role of the DNA damage response in our cancer cells, mice will undergo whole body irradiation and culled within 24 hrs.
To be able to switch on inducible genetic elements in cells that have been engrafted into mice, we may feed animals doxycycline food.
An important part of this project is the test of small molecule inhibitors to target proteins, either alone or in combination with known therapies. The route of administration may vary depending on the agent used. Animals may undergo multiple rounds of IP, IV or SC injections, or by oral gavage. To monitor any adverse effects in mice we will test blood samples for anaemia and leukopenia. In some cases, mice will undergo “drug holidays” and aged to observe if tumours are growing back.
For many of our experiments, 1-3 hrs before culling, mice will be IP injected with BrdU enabling proliferation rates of tumour to be determined in vitro.
Mice will be humanely killed at the end of experiments. Afterwards, we will analyse their tumours and organs in order better understand tumour development and how the drugs used worked.

What are the expected impacts and/or adverse effects for the animals during your project?

Many of the mice we are using have mutations which genetically predisposes them to generate tumours. Some of the animals will be engrafted with mouse cells that will form tumours. The main expected adverse effects will be related to this. Potentially, this could include weight loss (maximum of 20%), a reduction in BC score of <2, reduced activity, failure to respond to gentle stimulation, lethargic, abdominal distension, jaundice, piloerection, intermittent hunched posture, diarrhoea, or intermittent laboured respiration. Most of those symptoms usually appear at the late stage of tumour development.
When mice present with these symptoms the animal will be humanely killed immediately, so they will not experience them for long.
It is possible that delivery of chemotherapeutic drugs will cause adverse effects. As the drugs we will use are well reported in the literature, we will be able to pay close attention to the development of any signs of distress. For example, anaemia and leukopenia are common side effects of chemotherapy, and we will be able to monitor the extent of this by tail vein blood analysis.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most of our animals used for breeding carry a mutation which predisposes them to develop tumours. These animals will only be used for a limited period at a young age to minimise the possibility of tumour growth and will be killed immediately a tumour is apparent. 100% of animals may experience mild severity due to sampling for genotyping.
When ageing the animals with different mutations some (80%) may develop tumours. Animals that do not develop tumours will be classified as mild (as they have undergone at least one procedure), animals that do develop tumours will be classified as moderate.
In our implanted tumour experiments, control mice are expected to generate tumours as we need to compare

them to mice receiving shRNA or genetic material we hope will restrict tumour growth. Although all mice will undergo injection with the aim of developing tumours, genetic manipulation may slow growth rates and hence reduce severity experienced. We predict that 80% of mice will experience moderate severity.
Similarly, we are expecting that some small molecule inhibitors or their combinations will slow down or prevent tumour growth, unfortunately, it is unlikely that this will happen in all animals, plus a control group are needed with receive sham or vehicle, rather than the active compound. All animals that will be implanted with tumour pieces will be classified as moderate as this is a surgical procedure. Animals injected with cells will be classified as mild. Taken together 90% of animals will experience moderate severity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The majority of our work has used non-animal alternatives. Using mouse and human cell lines we have defined separation of function mutations that delineate specific molecular roles of BRCA1, and its binding partner BARD1. Similarly our findings of novel support pathways for BRCA1 mutants has arisen from non-animal methods.
In a partial replacement we will use cells derived from embryos, reducing the need for molecular and pathway analysis in the animals.
However, these means do not give an understanding of whether the genetic alterations (and subsequently altered molecular pathways) lead to growth of tumours or whether possible treatment approaches are likely to have in vivo treatment efficacy.
Very few, if any, in vitro cell culture based models are able to recapitulate the complex interplay between cancer cells and the tumour microenvironment. Indeed, studies using murine models of cancer that enable the modelling of disease within a complex multicellular living organism have been fundamental in enabling a better understanding of the processes that lead to malignancy that could not have been achieved otherwise. As a direct consequence of this, new drug targets and insights into the molecular mechanism of disease have come to light.
We need to use cancer tissue and cells in a live organism to evaluate the effectiveness of targeting a specific molecular targets on cancer development of real patient tumours.
Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.

Which non-animal alternatives did you consider for use in this project?

The majority of our work has used mouse and human cell lines where we have defined the separation of function mutations and identified of novel support pathways for BRCA1 mutants, while in a partial replacement we will use cells derived from embryos, rather than animals themselves.
In vitro data obtained from cell culture approaches will always guide in vivo studies, and we always conduct cell culture based experiments to justify the need to use animals. These systems include culturing cells in a

monolayer as a homogeneous population on plastic, and the more sophisticated 3D culturing of either cells lines grown in matrigel (as acinar) or mouse/human derived tissue as organoids. However, these models are not suitable to investigate the tumour development and how treatments affect it as mentioned in the above and below section.

Why were they not suitable?

There are significant limitations of basic culture conditions using cells grown in isolation as a monolayer on a piece of plastic as this is not an accurate representation of what occurs within a patient. Indeed, the reason why many new drugs fail between cell culture and in vivo studies is in the inability to full recapitulate the in vivo environment. Technologies are being developed to address this gap, including the development of 3D cultures (acinar cell line cultures and organoid mouse/human derived tissue). However, none of these model systems are yet able to phenocopy the integration and interplay between the numerous cell types that constitute the tumour and its microenvironment, or the fact that tumourigenesis occurs in and is influenced by biological systems (such as the immune system).
Moreover, genetic manipulation of organoid cultures is still technically challenging. Modelling cancer in mice is thus still required to fully understand disease progression and identify novel therapeutic avenues.
The development of 3-D tissue culture of tumour cells bearing the specific genetic changes we require risks losing the precious patient material, as many of the tumour and non-tumour microenvironment cells do not survive. Nevertheless, this is an avenue we are eager to continue to investigate. The advantage of in vivo propagation is it may allow us to increase the tumour material to test alternative means of 3D culture and organoid development.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For breeding, we have estimates of animal numbers based on our previous experience and number of strains which we will breed during the term of this license.
We have based the numbers of animals we will use for each experiment through consultation of the literature, in which experiments done in the past have assessed spontaneous tumour development and examined genetic changes that give a similar effect to those we are examining. These allow us to work out how many animals per group are likely to be needed to detect a difference, for example in tumour growth. Our estimates also take into account how many different strains and strain combinations we intend to examine.
For treatment approaches our numbers are based on how many animals we will need to detect an effect, for example of a drug, of a 25% reduction in tumour growth. These numbers similarly take into account how many methods we intend to study.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will use the EDA in conjunction with a local statistician to assist with experimental design to reduce animal numbers, methods to reduce subjective bias, and appropriate statistical analysis without compromising the scientific objectives. When possible, experiments will involve a factorial design that will maximise the information

obtained from a minimal number of animals. For example, non-invasive imaging and quantification techniques of transplantations will enable multiple measurements on the same animal over a period of time. In such cases, ANOVA will be utilised for statistical analysis.
When conducting engraftment experiments, we routinely inject two contralateral flanks of the mouse, thereby reducing the number of animals being used by a half.
Our approach of modelling breast cancer by induction with oncogene injections will substantially reduce the number of animals used in experiments as most of those animals will develop tumours and the need for lengthy breeding is reduced.
Where possible, we will use live imaging to track tumour development longitudinally. Not only does this mean fewer animals are needed overall as there is no need to cull at each time point, but it also reduces variation and so improves the quality of the data produced.
Two flank sites or two intraductal injections will be used only when control and experimental material can be introduced into a single mouse. This approach reduces the number of mice used.
Strains not immediately required for the scientific study will be cryopreserved as embryos.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies to estimate variability and perform power calculations to calculate sample sizes.
Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.
We always strive to generate the most effective breeding strategies to ensure that we obtain mice of the desired genotype with minimal animal wastage. If we are unable to estimate an effect size from our in vitro data, the literature, or our collaborators, we will conduct small pilot experiments.

All tissue surplus to requirement will be deposited into SEARCHBreast (https://searchbreast.org), a resource to facilitate sharing of archived material derived from in vivo breast cancer models.
In vivo imaging of engrafts will be conducted to enable us to monitor tumour progression in living animals throughout the experiment and will therefore reduce animal numbers by performing longitudinal measurements in fewer animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are the species of choice for a genetic approach to the analysis of biological phenomena. In particular, the wide availability of genetically modified mouse strains and mouse ES cells means that the function of most genes of interest can be studied in the tissue of choice.
We have mouse models which carry mutations found previously in patients with breast cancer that will develop tumours with similar aetiology and molecular profile as that observed in human disease. Hence, we will be aware of potential adverse effects and monitor for them appropriately. In line with this, we will use tailored welfare sheets that have been developed based on our experience with other strains. The phenotype of any novel genetically modified strain that we generate will be closely monitored for signs of distress and handled in the appropriate manner and a tailored welfare sheet developed.

We plan significant refinement by using intraductal delivery of viral particles containing oncogene. This procedure has recently been used in many laboratories and requires fewer animals than traditional our models. By using this procedure we are considering Reduction and Refinement aspects, thereby enhancing the welfare experience of mice used during our experiments.
To decrease tumour events in our breeding colonies we will keep young animals for further breeding.
In vivo imaging of engrafts will be conducted through the use of non-invasive techniques that will enable us to monitor tumour progression in living animals throughout the experiment and will therefore reduce animal numbers and achieve better humane endpoints. Here, the tumour volume as determined by non-invasive caliper measurements or bioluminescence imaging will be plotted against time. This design offers the advantage of determining significant differences between tumourigenic growth potential of cell lines before the limited tumour volume is reached. We will use immune compromised mice in order to achieve engraftment. These models are characterised in pilot studies to identify any unexpected adverse effects.
For testing a new treatment strategies, we will perform pilot studies to determine dose range and develop score sheets and robust human endpoints.

Why can't you use animals that are less sentient?

We need to study tumour development in adult mice as this is what most closely resembles what occurs in humans. We have chosen to use mice over other less sentient species such as Danio Rerio (zebra fish) and drosophila melanogaster (the fruit fly) as mice and humans share 97.5% of their coding DNA sequences. In comparison, the drosophila genome is only 60% homologous to that of humans and only
75% of the genes responsible for human diseases have homologs in flies. Mice are also more appropriate for studying complex biological systems found in humans as they possess immune, endocrine and nervous system. Consequently, like humans, mice naturally develop diseases, including cancer.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Through advice of colleagues performing similar work we are aware of refined treatment doses and route of administration for four of our most commonly used anti-cancer drugs so that we know the maximum tolerated dose, the minimum effective dose, and sub-optimal doses. This minimises the possibility of adverse effects from chemotherapy.
Animal welfare is a key consideration in all of our protocols and we will be guided by our NACWO and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. Following every experiment and regularly during group meetings we will review our procedures from a welfare standpoint to identify any potential for refinement.
When conducting a surgical procedure, we ensure that analgesia is administered 30 min before the start of the procedure. Mice are allowed to recover from the surgery by being housed in a warm cage and observed by a member of the team until the animal has fully recovered and is mobile. Mice are checked again for wellbeing and wound closure 4hrs later. We have found that by placing the wound towards the bottom third of the spine with a combination of suturing and glue, mice are less likely to bite and cause reopening.
Once mice start to develop tumours, they are monitored at least twice a week, and more so if tumour growth develops rapidly. Tumours that developed in the mammary gland are carefully monitored for signs of ulceration, and hard housing replaced by cardboard housing and additional bedding if this occurs.
We are fortunate to have excellent colleagues both within our local research community and our animal facility, who have extensive, relevant animal procedure experience from whom we can learn refined techniques from. Our team will undergo extensive training on dead animals and require to be authorised as competent by the NTCO before being allowed to perform a procedure on live mice.
Examples of refinement;
Repeated injections (therapeutics) will be done on opposite sides or at different areas from previous injection so as to not aggravate any visible bruising.

In order to reduce the possibility of ulceration where external tumours are present, hard cage environmental enrichment (e.g. plastic houses) will be replaced with additional nesting material and cardboard houses. If animals are showing early signs of ill health then long spouts will be supplied on drinking bottles. Animals comfort and ability to move and reach food/water will be monitored daily.
Intraductal injections into the 4th mammary gland avoid the 1st gland as tumour growth may impede movement.
Use of fractionated irradiation scheme and antibiotics in the drinking water will significantly reduced adverse effects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidelines for the welfare and use of animals in cancer research. Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA; Committee of the National Cancer Research Institute. Br J Cancer. 2010 May 25;102(11):1555-77. doi: 10.1038/sj.bjc.6605642.
RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)

Jones HRP, Oates J, Trussel l BA (1999) An applied approach to assessment of severity. In: Humane End points in Animal Experiments for Biomedical Research (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 40±7.
We aim to publish in journals that support the ARRIVE guidelines and conduct our experiments with advice from the PREPARE publication (PREPARE: guidelines for planning animal research and testing. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.PMID: 28771074).
Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.
We will use SyRF the free online platform for researchers to perform a systematic review and metaanalysis of animal studies. https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will comply with the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments; www.nc3rs.org.uk/arrive), a NC3Rs-developed checklist of the essential information that should be included in publications reporting animal research. ARRIVE has now been endorsed by more than 400 journals including the Nature group, PLoS, and Cell, as well as funders, universities, and learned societies.
I also monitor the NC3Rs twitter account, so will be made aware of any notification via social media.
Any new advancements will be made clear to members of my team through our weekly lab meetings.
Literature searches, attendance at vendor’s information sessions, seminars and conferences to find out about new technology and new approaches that we could implement.

Home Office

NON-TECHNICAL SUMMARY

18. Cancer Vaccine Development Using Immunotherapy by Natural Anti-Carbohydrate Antibodies

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim is to provide in vivo proof-of-concept of the tumour vaccine approach by demonstrating efficacy in mice where circulating antibodies have been raised.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Around 1 in 2 people in England develop cancer in their life-time, with cancer now causing around 1 in 4 deaths. This picture is similar throughout the 'developed' world. There is thus a clear need to develop new therapeutic strategies for cancer, to continue the progress seen in the past 20 or so years due to the movement towards targeted therapies, with improved survival rates seen for most common solid cancers.

There is a growing interest in immunotherapy as a treatment for cancer, and there are several strategies which have been adopted for this which have successfully progressed to the clinic, e.g. Rituximab for NHL and Atezolizumab for bladder cancer. Despite the progress, however, clinical studies have raised two fundamental challenges. 1) The majority of patients still do not respond to the molecular blockade at the heart of the therapy. 2) The mechanism of action of the therapy has consequently been found to lead to a set of undesired immune- and autoimmune-related adverse events, which observation indicates a broad-range of side-effects mainly occurring at barrier tissues (e.g. the skin, gut and lung). Therefore improved clinical practices are urgently needed to overcome the limitations of tumour heterogeneity and inaccuracy for immunotherapy.

A company we are collaborating with focuses on an alternative novel approach to overcome these limitations and supplement immunotherapy using a highly effective carbohydrate-to-protein ligation probe based on two criteria:

1) It selectively, yet homogenously, labels a person's own tumour cells with immunogenic carbohydrate moieties, thus providing a tumour and person-specific treatment opportunity.

2) It exploits the presence of circulating natural antibodies. These antibodies often recognize carbohydrate determinants, such as blood group antigens, so deploying them in an ex vivo ligation approach offers a safe and effective opportunity for intervention. The resulting immune recognition of autologous TAAs allows for the development of long-lasting personalized vaccines that may limit remission.

What has not yet been done is in vivo proof-of-concept studies, and these form the basis of this licence.

What outputs do you think you will see at the end of this project?

The therapeutic strategy which forms the basis of this licence has the potential to overcome the current challenges with 'conventional' immunotherapy, and the in vivo proof-of-concept studies carried out under this licence will validate the potential of this approach, and allow further progress towards the clinic through late-stage preclinical and clinical trials.

Who or what will benefit from these outputs, and how?

Ultimately the hope is that the therapeutic approach being investigated here will translate to the clinic and benefit cancer patients. This will not be until beyond the timeframe of this project, with perhaps another 5-10 years of preclinical and clinical development required to realise this aim.

How will you look to maximise the outputs of this work?

As the work is being carried out on behalf of a biotech company, then dissemination of any positive or negative information will be their decision.
Where we have any information that we can use that is non-confidential, then we will use this on our website and associated material in order to promote the work as a potential service going forward.

Species and numbers of animals expected to be used

- Mice: 1600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the most frequently used mammalian species for cancer immunotherapy studies. This is beneficial when carrying out such studies due to the depth of knowledge available plus standard protocols, methods and reagents which have all been optimised for use in this species. In addition it is important to use a mammalian model, since this closer models for the components of the mammalian immune system seen in the clinical situation, which would not be the case if a different Class is used.
Adult animals are used since these will have a fully developed immune system.
Typically, what will be done to an animal used in your project?

Each protocol covers different facets of the project, with some of the experimental steps similar across the project.

In protocol 1, once a suitable candidate immunogenic carbohydrate has been identified through extensive preliminary in vitro work, then the initial work in vivo will be to demonstrate that it is possible to introduce this antigen (which is normally not present in mice) to immunocompetent mice and generate antibodies to it. This will involve injection of the antigen to generate antibodies, and blood sampling to check for antibody titre. Typical duration will be ~2-3 months.
In protocol 2, tumour cells will be inoculated s.c., and the primary tumour grown to a volume of approximately 300mm3 as measured by callipers. The animals will be killed and the tumour will then be excised and vaccine prepared by isolating the tumour cells ex vivo and labelling with the modified antigen. Typical duration will be ~2 months.
In protocol 3, following confirmation of generation of circulating antibodies, primary tumour development will be monitored after s.c. inoculation of tumour cells. After measurable xenograft growth is evident, a tumour vaccine either comprising inactivated and homogenised in vitro cultured or in vivo-derived (P02) tumour cells labelled with the modified antigen will i.v. be administered. Calliper measurements will be utilised to assess tumour volume. To further confirm efficacy is down to the immunotherapy, systemic effector T cell responses will also be

assessed, by isolating T cells from harvested spleens, and evaluating expression of markers such as cytokines. Typical duration will be ~3-4 months.
In protocol 4, further complexity is added compared to P03, by monitoring efficacy in a model of tumour dissemination. The in vitro or in vivo derived vaccine will be administered to mice, followed by i.v. inoculation of tumour cells, and monitoring of the effects of the presence of vaccine on generation of lung tumour deposits. The number of lung deposits will be assessed by both counting of surface deposits in fixed excised intact lungs, and by histological evaluation, and where a fluorescence or luciferase tag has been engineered into the cells then tumour burden can also be monitored noninvasively. To further confirm efficacy is down to the immunotherapy, systemic effector T cell responses will also be assessed. Typical duration will be ~3-4 months.
In protocol 5, the potential of this therapeutic strategy to enhance the efficacy of ICI therapies will be investigated. Here following confirmation of generation of circulating antibodies, and administration of vaccine, after i.v. or s.c. tumour inoculation, then an appropriate ICI therapy will be administered, e.g. anti-CTLA-4 immunotherapy. As above, generation of lung tumour deposits for i.v. inoculation, or s.c. inoculation of the tumour cells and monitoring the effects on primary tumour growth will be carried out, and to further confirm efficacy is down to the immunotherapy, systemic effector T cell responses will also be assessed. Typical duration will be ~3-5 months.

What are the expected impacts and/or adverse effects for the animals during your project?

For protocols 1, 3, 4 and 5, Freund's Complete Adjuvant can be an inducer of an intense local inflammatory response at the site of injection. We envisage an incidence of <1% for this occurring. We might expect this effect to last for no more than a few weeks.

For protocols 2, 3 and 5, potential harms would be the tumour becoming sore, inflamed, infected or ulcerated, or approaching a maximum combined volume per animal of 1200mm3. Any showing signs which would suggest they are likely to exceed moderate severity are to be killed by a Schedule 1 method. It would be expected that <1% of animals would experience these symptoms for no more than 72 hours.
For protocols 3, 4 and 5, one might see inflammation at the sight of injection of luciferin. There may also be a systemic reaction to administration of the anaesthetic. It is predicted that both these events will occur with a <0.1% frequency. We might expect the inflammation to last for no more than 72 hours, with the anaesthesia effect a matter of hours.
For protocols 4 and 5, tumour burden in the lungs may have an impact on lung function as evidenced by abnormal breathing and panting. From previous experience, <1% of inoculated mice experience these symptoms and would be sacrificed if this persisted for 48 hours.
In addition to the specific harms detailed above, in general, animals are checked twice daily by competent animal care staff who will notify the PIL holder and/or NACWO of any indication of pain, suffering or distress including but not limited to abnormalities in behaviour and/or appearance, such as poor coat condition, piloerection, unusual posture (hunching), discharge or lack of grooming to eyes, ears, nose, ano-genital region, facial grimace, obvious signs of injury, abnormal movement, reduced activity, reluctance to socialise, reduced alertness, vocalisation, indications of reduced eating or drinking. An informed assessment of the level of severity will be made based on the above observations, the known phenotype, and the procedures undertaken and appropriate treatments/ actions such as antibiotics, analgesics, or euthanasia will be considered (taking NVS advice where needed).
In cases where unexpected side effects are observed which are expected to exceed the moderate severity limit, the animal will be humanely killed by a competent person. Indications that a moderate severity limit is likely to be exceeded include but are not limited to, significant (easily detectable by a competent person) or lasting (more than a few hours following procedures, or 48 hours in stock mice) changes as listed above.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

It is expected that the majority of animals (~99%) will experience no more than mild severity, with ~1% experiencing moderate severities of the types described in the section above on expected impacts/adverse effects.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

There are 2 key reasons why it is necessary to use animals in order to evaluate the efficacy of the immunotherapy strategy under investigation here.
Firstly, there is the multi-faceted immune response which is stimulated by the presence of the vaccine. Whilst in vitro it might be possible to assess one or a few components of the immune system response, what is not accounted for is the complex interplay between the various components which can be generated in different locations in the body in response. Therefore an intact immune system is required to follow all these.

Secondly, as has been seen with the lack of success with ICI-based therapy, issues have arisen with off-target immune and autoimmune effects occurring in a range of tissues and organs, and therefore a whole body approach is required to validate the desired therapeutic effects. The off-target effects would manifest at the gross level in the deleterious effects we would be monitoring for throughout the procedures.
As mentioned above, extensive preliminary in vitro investigations will take place to ensure every chance of success with the selected target antigen in vivo.

Which non-animal alternatives did you consider for use in this project?

For efficacy, none, for the reasons given above **Why**

were they not suitable?

See above

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This is based on estimate of number of antigens / vaccines that will be evaluated over the course of the project, with the numbers of animals for each particular study calculated by power calculations where necessary. For each protocol this breaks down as follows over the course of the project: P01 ~20 tests; P02 ~50 tests; P03 ~15-20 tests; P04 ~10-15 tests, and P05 ~ 5 tests.
In order to determine the minimum numbers of animals required to demonstrate a statistically significant control of tumour compared to controls, the B16 lung deposit- and the subcutaneous melanoma models have been used as these will be the most commonly used models in these studies.
To determine the expected range of metastatic nodule numbers in the lung and melanoma xenograft growth in the B16.F0/F10 models, fifty scientific publications have been carefully examined for each, and we have carried out statistical power analyses based on this data.

These calculations indicate that for an effect of of the minimal desired reduction in tumour development, the investigators need to work with a minimal sample size of n = 9-12 in both models. Therefore, group sizes of 10 animals are proposed for all work on P03-P05, which also take into account an experimental dropout rate of at least 10 %.

If we work with other tumour models, then a similar exercise will take place to determine the minimally significant group size required.
Animals will be randomly assigned to experimental groups at random within a cage. Due to the aggressive and territorial nature of some strains of mice which could be used, we will avoid mixing cages to form the groups.
For sample analysis where feasible, samples will be blinded.
We will also consider using the Experimental Design Assistant for planning experiments available through the NC3Rs web-site, and refer to the ARRIVE Guidelines (Animal Research: Reporting In Vivo Experiments) on reporting animal experiments to ensure relevant endpoints when designing individual experiments to further aid in minimising animal use.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Discussion with scientists at the company we are working with and reference to the literature
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The use of pilot studies as mentioned elsewhere.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and

methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are the most frequently used mammalian species for cancer immunotherapy studies. This is beneficial when carrying out such studies due to the depth of knowledge available plus standard protocols, methods and reagents which have all been optimised for use in this species. In addition, it is important to use a mammalian model, since this closer models for the components of the mammalian immune system seen in the clinical situation, which would not be the case if a different Class is used.
If the cells lines that are being used have been engineered to express a fluorescent tag, e.g. Green Fluorescent Protein, or luciferase, then this gives the capability to perform non-invasive optical imaging (fluorescence or bioluminescence) and allows us to monitor internal tumour burden throughout the ongoing experiment. This minimises the risk of developing an unexpectedly high tumour burden, as we can follow tumour growth 'live'.
Animal suffering will be minimised by adhering to the UK National Cancer Research Institute Guidelines for the Welfare of Animals. Specifically, we will always use aseptic technique and will commit to the LASA Guidelines as set out in 'Guiding Principles for Preparing for and Undertaking Aseptic Surgery' (2nd edition, April 2017).
Analgesics and anaesthesia will be used where required, and we will take advice relating to care and welfare via the AWERB and other appropriate sources.

Why can't you use animals that are less sentient?

It is important to use a mammalian model, since this closer models for the components of the mammalian immune system seen in the clinical situation, which would not be the case if a different Class is used.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Analgesics and anaesthesia will be used where required, and we will take advice relating to care and welfare via the AWERB and other appropriate sources.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Animal suffering will be minimised by adhering to the UK National Cancer Research Institute Guidelines for the Welfare of Animals (ref). Specifically, we will always use aseptic technique and will commit to the LASA Guidelines as set out in 'Guiding Principles for Preparing for and Undertaking Aseptic Surgery' (2nd edition, April 2017).
For blood sampling we will follow guidelines as set out in the 'Handbook of Laboratory Animal Management and Welfare, Third Edition' Editor(s): S Wolfensohn & M Lloyd (2003) & use as a a guideline for clotting time, Emeis et al. (2007), J Thromb Haemost, 5: 670-679.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through scanning most recent literature, referring to the NC3Rs website and through information passed on by the local NIO.

Home Office

NON-TECHNICAL SUMMARY

19. Cell plasticity in the normal and diseased brain

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

brain cancer, stem cells, neurological disease, pathophysiology, epithelial-mesenchymal transition

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our research is focused on understanding biological mechanisms of cell plasticity in the central nervous system. The main goal is to identify regulators of cell plasticity that are relevant for the development and progression of neurological insults, such as traumatic injury, neurodegeneration and brain cancer.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cell plasticity is an important process in tissue maintenance, tissue repair and the response to injury, as well as in the development and progression of diseases. Cell plasticity allows certain cells to adapt to a changing environment and to dramatically alter their gene expression and phenotypes. Alterations in cell plasticity occur during normal development and tissue homeostasis, e.g. in specification of cell lineage, but also accompany various neurological insults. Many of these neurological insults cannot be treated appropriately, resulting in a major burden on healthcare systems and significantly impacting the lives of patients and carers (e.g. traumatic brain injury, neurodegeneration such as Alzheimer's disease, Parkinson's disease and Huntington's disease, and brain cancers).
Our research focus is to understand the cellular and molecular biology of cell plasticity in the CNS, and how this contributes to normal brain function as well as to neurological insults. In many neurological diseases, changes in cell plasticity either directly or indirectly affect the progression of the disease and/or the response from healthy cells. A better understanding of how molecular regulators of cell plasticity act in the normal brain and how their functions are altered by disease processes is essential to develop new and improved therapeutic options for these devastating diseases.

What outputs do you think you will see at the end of this project?

This project will generate new information in the biology of EMT-AMs and how these molecules regulate cell plasticity and lineage selection in health and neurological diseases. We expect to increase understanding of fundamental biological processes that are broadly applicable to stem cells and their differentiation. This will reveal targetable vulnerabilities that can be leveraged to develop new treatments for neurological diseases that are currently incurable. For instance, our work will evaluate the contributions of reactive astrocytes to traumatic injury, and neurodegenerative diseases (e.g. Huntington's disease, Alzheimer's disease) and how modulating this reactivity impacts on disease progression. We will further probe how cell plasticity contributes to brain cancer progression and how blocking this can be used for treating brain tumours. Finally, increased understanding of how EMT-AMs regulate brain cell plasticity may also be beneficial for regenerative medicine approaches, e.g. in areas of stem cell transplantation.

Who or what will benefit from these outputs, and how?

In the short-term, outputs such as new information and experimental models will benefit the neuroscience, stem cell biology and cancer research communities. The clinical benefits of this work are likely to be long term; our principal hope is that by investigating regulation of cell plasticity, we may identify new targetable vulnerabilities in neurological diseases (injury, neurodegeneration and brain cancer) that can be exploited for the development of new therapies. It is noteworthy that during our work under the previous PPL, we successfully identified a new, targetable pathway that may be exploited to treat the brain cancer, glioblastoma.
The project also contributes to the refinement of existing animal models of brain cancer to better mimic the

clinical setting. This will improve the relevance of experimental findings and thus further increase the likelihood that new therapeutic strategies will successfully be applied to humans.
Given the ubiquitous expression patterns of EMT-AMs, the putative therapeutic targets identified here may also be relevant to other tumour types, and therefore may have impact on a broader range of clinical settings, including other cancer types.
Additional long-term clinical benefits may arise from evaluating regulators of cell plasticity for regenerative medicine approaches. Great effort in this field is dedicated to the guided differentiation of specific cell types for transplantation. Our work may help to design protocols for guiding differentiation into specific cell types.

How will you look to maximise the outputs of this work?

All knowledge generated in this project will be disseminated through peer-reviewed publications and presentations at national/international conferences/scientific meetings. Impact will be maximised through press releases accompanying publications and distribution on social media to alert interested stakeholders. Data will be shared via open access journals and repositories. We will continue to actively engage with the general public via events within the University/local communities and via national charities. Other progress and successes in key milestones will be released via University research pages, newsletters and social media.

Species and numbers of animals expected to be used

- Mice: 6000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use genetically altered mouse models of EMT-AMs and CNS cancer and primarily juvenile and adult life stages (including aged animals; e.g., > 26 weeks old). Mouse models recapitulate human disease accurately at the genetic, tissue and clinical level. Our research goals are to investigate functions of EMT-AMs in the development and progression of CNS cancer and neurological diseases. By using genetically altered mouse lines and Cre-lox technology, we will determine genetic predisposition in specific target tissues and unravel whether/how exogenous risk factors such as inflammation contribute to disease. All mouse lines used in this project will also express a reporter (e.g., fluorescent protein), which will allow us to monitor and assess the fate of mutant cells in tissues over time.

Typically, what will be done to an animal used in your project?

Typically, we induce conditional gene expression/deletion in juvenile/adult mice (e.g., 4-8 week old) using inducible-Cre recombinases and under the control of a CNS-specific promoter, e.g., tamoxifen induced GLAST CreERT2. Expression of Cre recombinase also induces expression of a fluorescent protein (e.g., tdTomato) from the ROSA26 locus. Induced animals are aged to a defined end point (e.g., 4 or 8 weeks post induction) before being killed by a Schedule I method or transcardial perfusion.

What are the expected impacts and/or adverse effects for the animals during your project?

We expect that expression of oncogenes such as KrasG12D combined with loss of tumour suppressors (e.g.

Tp53, Pten) in the CNS will lead to the development of tumours over time. In these cases, adult animals may experience the adverse effects of malignant tumours such as weight loss, hunching, inappetence, lack of grooming. Since we are interested in tumour development and progression, we will not routinely use tumour burden as an end point and experiments will be stopped before the appearance of clinical signs in many cases. Neurological diseases, such as neurodegeneration and brain injury, will impact animal health. Animal models of neurodegenerative diseases, or animal models with brain injury, may experience adverse effects from progressive loss of neurological functions, which manifest by weight loss, hunching, lack of grooming, and potential motor symptoms (e.g. increased or decreased motility). These symptoms occur in later stages of neurological disease. Clearly defined criteria for monitoring animal health ensure that animals will not experience symptoms longer than necessary.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect tumour development to have a moderate severity. Only animals that carry site-specific Cre recombinases and a combination of conditional alleles will develop tumours. CNS tumorigenesis requires the combination of multiple alleles (e.g. loss of Tp53 and Pten, and expression of mutant KRas G12D). Based on current experimental design, approximately 25% of cohorts may experience a moderate severity (e.g., GLAST-CreERT2/+; KrasG12D/+, Tp53 f/f, Pten f/f animals).
Animals exhibiting symptoms of neurodegenerative diseases are expected to have a moderate severity. Based on experimental design, approximately 25% of cohorts may experience a moderate severity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Several aspects of neurological diseases, such as brain cancers, neurodegeneration or brain injury, cannot be feasibly studied outside of the living organism. Development and progression of neurological diseases are critically influenced by interactions between different brain cell types. Yet, there are currently no in vitro or animal-free simulations that allow us to study the complex and multifaceted interactions of all of the interconnected cell types at play within the living organism.
There are most certainly, reductionist, cell-culture models of specific aspects of neurological diseases within the literature; however, none of these systems can faithfully recapitulate all aspects of neurological disease. Essentially because these in vitro systems are intentionally simplified, they do not allow for the degree of complex cellular interconnectivity required for the proposed studies.

Which non-animal alternatives did you consider for use in this project?

Ex vivo explant models offer non-animal alternatives for some aspects of this work but are limited.

Why were they not suitable?

Current ex vivo CNS explant models do not robustly recapitulate healthy normal tissue, principally because CNS tissue is complex, composed of multiple cell types that are exquisitely sensitive to manipulation outside the organism. Essentially, growing neurons or glial cells derived from later than early postnatal stages is impossible.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers were estimated based on previous experience and then validated using the NC3Rs Experimental Design Assistant and Power Calculations. For these calculations, effect size (m1-m2) and variance (S.D.) were calculated using current data, or from data reported in the literature.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We calculated minimum group sizes to obtain statistically significant data from our planned studies. All calculations were made using the NC3Rs Experimental Design Assistant and with data from our own research or from published literature to estimate variability and effect size. This allows us to reduce the overall number of animals used for experiments.
Wherever possible, experiments are designed to avoid redundancy by addressing multiple questions (e.g. tissue from animals used for behavioural studies will also be used to analyse histology).
Wherever possible, experiments are designed so that one cohort of control animals can be used for multiple cohorts of experimental animals to avoid redundancy.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

All aspects of this work will be supported by experiments involving cells isolated from brain tissues and tumours and maintained in culture in the laboratory. This will reduce the number of animals required. However, animals are required to study tumour biology because it involves a complex interaction between multiple tissue types and the immune system, therefore analysis of tumour behaviour or neurological diseases ultimately requires intervention in the context of the whole organism. Determining overall cohort sizes is based on previous experience with these models and validated using power analyses. The use of non-invasive imaging techniques, while part of the research and development of new diagnostic tools in their own right, will further reduce animal numbers. Where appropriate, homozygous parents will be bred to reduce numbers of excess offspring of incorrect genotypes.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use genetically altered mouse models and site-specific Cre-lox technology that allows temporal and inducible gene modification in CNS tissue. Mouse strains will also express reporters of Cre that will allow tracing of recombined cells in tissues over time. All genetically altered lines will carry conditional alleles; therefore, only mice that carry site-specific recombinase and the conditional allele will develop tumours. These models are well characterised during work undertaken under the previous PPL and by our collaborators; experimental time points and timelines of the development of predictable phenotypes is known. We will only use genetically altered animals appropriate to our objectives (i.e., targeting of specific transgenes in a tissue-dependent manner) to ensure that the work carried out is accurate. In vivo imaging approaches (where appropriate) provide non-invasive methods to monitor disease progression in the same animal over time.
Tissue transplantation assays provide approaches where we can uncouple cell intrinsic from cell extrinsic factors in vivo; test putative cancer stem cells and monitor tumour progression in short-term assays without the need for primary tumour formation.

Why can’t you use animals that are less sentient?

Mice are considered the most suitable experimental animal model for neurological diseases, including brain cancer, neurodegeneration and brain injury, for the following reasons:
There is a large body of knowledge on the physiology, histology and molecular biology of the mouse CNS; there are a wide range of existing genetically modified lines, a number of which are directly relevant and amenable to our studies; the relatively short life-cycle and high fecundity of mice is advantageous for genetic studies; and mice are generally regarded as being of lower sentience compared to other mammals such as the primates.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinements will include frequent monitoring of induced, post-operative or anaesthetised animals. Exogenous substances/agents will be administered at the minimum dose, as determined using NC3Rs guidelines for best practice and dosing regimens and following consultation with our collaborators. All procedures will be carried out by trained and experienced staff with the support of experienced animal facility staff/NACWOs/NVS (e.g., application of anaesthesia, analgesics, surgical techniques).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We have extensive experience in all procedures from work during the previous PPL. All procedures will follow LASA guidelines and we will make sure to implement any updated recommendations on best practice.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Engage with NC3Rs representatives on a regular basis via local workshops/meetings, newsletters and social media.

NON-TECHNICAL SUMMARY

20. Central Nervous System (CNS) Safety and Efficacy in Rodents

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Mental illness, Central Nervous System, Mood disorders

Animal types	Life stages
Mice	adult, pregnant, neonate, juvenile
Rats	adult, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to support the assessment of a test agents for potential adverse effects on behaviour or their ability to modify impaired mood, sensation, thinking and behaviour.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Mental illness is a growing problem, with 1 in 4 people experiencing mental health issues each year, with an associated economic and social burden.

- Depression is a leading cause of disability worldwide, often occurring in conjunction with other mental health issues and associated with suicide and heart disease.
- Anxiety affects a significant number of people, and over recent years has been seen to be increasing among young people.
- Psychosis and schizophrenia are also on the increase, with the latter being increasingly connected to cannabis consumption at an early age.
- Autism Spectrum Disorders (ASD) is increasing. ASD is complex and is associated with varying levels of disability, including those related to social interaction, repetitive behaviours, frustration, aggression, anxiety, sensory sensitivity and motor coordination. In addition,
  ASD commonly co-occurs with other diagnoses including developmental, psychiatric and neurologic conditions.

Such conditions can be hugely debilitating for the individuals and challenging for care givers and family.
Although a number of treatments are available for such conditions they are often associated with varying degrees of side-effects. For example, anti-psychotics may cause weight gain, suicidal feelings/behaviour, seizures, sedation, neuromuscular effects, emotional effects and heart problems. As such, new medications are required which have improved or equal efficacy with lower associated side-effects.
For the treatment of Schizophrenia, medications have primarily targeted the positive symptoms (e.g. hallucination, delusions, disorganization speech and behaviour), however, more recently, there has been an increase in drugs being developed to treat the negative symptoms of this condition (e.g. lack of emotion/hedonia, low energy, low motivation and impairment of social interactions).
Data from this project will be used to improve human health by 1) supporting the development of new medicines for these and other related disorders, 2) assessing novel drugs for their potential to produce unwanted behavioural/CNS side-effects and 3) support the requirements of regulatory submissions.

What outputs do you think you will see at the end of this project?

Reports and data which will be submitted to support the regulatory filing of new drugs.
The refinement of models and associated publications sharing such data and best practices.

Who or what will benefit from these outputs, and how?

The primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to informed decisions, based upon data generated in these studies, regarding the risks and/or benefits when humans are exposed to medicinal products.
Achievement of the objectives of this Licence will enable medicinal products to progress into clinical testing and onwards to marketing authorisation and ultimately improving the health and welfare of humans. Without these pre-clinical studies, progression of new medicines to early human studies and on to patients/marketing could not occur.
There is also a major benefit when using early drug selection type experiments, as the most promising compounds for further development will be selected and then fully tested so that they can reach the market sooner and as such benefit the health and welfare of humans.
The use of the experiments described in this licence may indicate major safety concerns or lack of effectiveness with the substance under evaluation at an early stage thus precluding requirement for additional experiments after these screening studies. This can greatly reduce the number of animals required in a programme of work. In addition, scientific knowledge gained in one programme of work will often be applied to future experiments in order to reduce animal numbers and/or reduce pain and stress to those animals used in the subsequent work, or to target investigations to a particular organ or tissue during toxicity testing or clinical trials.

Sponsors will benefit from work undertaken within this project licence, by obtaining data which allows them to make decisions on the development of the drugs and to support regulatory filings.

How will you look to maximise the outputs of this work?

Where confidentiality permits, data, study design and best practice will be openly shared at conferences, workshops, webinars, blogs and publications.

Species and numbers of animals expected to be used

- Mice: 3500
- Rats: 3500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Animals used during the course of this licence will be rodents. There is wide knowledge of the behavioural response of rats and mice in response to a wide range of substances and a wealth of background literature. The innate curiosity and anxiety of these species has established them as species of choice for assessing conditions involving emotional state.
Although the majority of studies will use adult animals, on occasion, neonatal or pregnant animals exposed to valproic acid (a medication for the treatment for various types of seizures) may be required for modelling autism disorders. In addition, on rare occasions, juvenile animals may be used when age-sensitive effects are of concern.

Typically, what will be done to an animal used in your project?

The majority of animals within this project will administered test agents as a single administration or daily administration for up to 4 weeks. At various time-points animals will be observed for physiological or behavioural changes (e.g. body temperature, anxiety, exploration, social interaction, learning and memory, reaction to a stimulus). Blood samples may be taken on occasion so that test agent exposure levels or biomarkers of disease can be determined and related to the human situation.
These assessments may be conducted in normal animals, animals genetically susceptible to emotion state disorders, or animals which have various emotional states induced chemically. Animals assigned to protocols which require them to learn to lever press in order to obtain a positive reward (e.g. sugar pellet) have an extended duration or several months which is largely determined by how quickly the animals learn the task.
In all cases, upon completion of testing the animals are humanely killed.
The endpoints will be carefully selected based on the purpose of the study and by considering the effect that each test may have on the outcome of another test or on multiple testing. In general, each observation (test) is of short duration (e.g. 15 minutes). Examples of study designs based on therapeutic purpose are as follows;

- **Positive symptoms of Schizophrenia could be assessed in amphetamine pre-treated animals**
  Locomotor activity
- Visual observation for stereotype behaviour
- Pre-pulse inhibition
  (acoustic startle)

Negative symptoms of Schizophrenia could be assessed in MK-801 pre-treated animals

- Novel object recognition
  Social interaction
- Morris water maze
  Anxiety
- Social interaction
  Holeboard activity
- Light/dark box
  Staircase test
- The above could also be applied to Autism Spectrum Disorder in genetically susceptible animal or animals previously exposure to valproic acid.
- **OCD**
- Marble burying
  Pre-pulse inhibition
- The above could be applied to Autism Spectrum Disorder in genetically susceptible animals or animals previously exposure to valproic acid.

In all cases, a single intravenous administration of a non-labelled tracer may be administered (e.g. 15 to 60

minutes before the animal is killed) for receptor occupancy purposes.

What are the expected impacts and/or adverse effects for the animals during your project?

Humane endpoints (documented within the licence) will be applied to animals used under the protocols specified in this licence.
Although these studies are typically conducted at the early stage of drug development and often for lead optimization purposes, preliminary toxicology and or pharmacokinetic studies are anticipated to have been performed and as such the dose levels can be carefully selected in order to avoid undue toxicity. The majority of the protocols within this licence are behaviourally based and as such adverse effects are not desirable.
These assessments may be conducted in normal animals, animals genetically susceptible to emotion state disorders, or animals which have various emotional states induced chemically. In the majority of cases, the resultant effects will not be obvious, with animals appearing normal. However, under certain conditions, such as a test which requires the animal to remember a task, to interact with another animal, respond to a novel environment or response to an auditory stimulus, the genetic or chemically-induced phenotype may be revealed. Such effects are also anticipated for positive controls used within this project licence.
If overt effects are seen, then these are anticipated to be transient and will typically be stimulant- or sedative-like.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The severity limit specified (moderate) is considered to be the minimum commensurate with achieving study objectives. For the majority of studies conducted under this licence, the severity limit is moderate due to the procedures required to achieve the protocol objectives. Repeat dosing and multiple subthreshold and mild procedures are primarily responsible for the moderate severity imposed. In the majority of cases animals will not experience more than mild signs as a result of administration of the test agents. Marked effects anticipated are primarily those related to the known pharmacology of drugs e.g. sedation or stimulation.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

As a service provider we do not own the test materials under evaluation, therefore, in silico screening tools for candidate selection are not appropriate, however, available literature is searched prior to commencing any in vivo procedures. Prior to conducting in vivo studies, sponsors are requested to provide information relating to their test material candidate, together with details of other work performed, relevant regulatory requirements and a justification for conducting in vivo investigations.
Due to the studies within this project requiring the assessment of behavioural and learned responses, the use of animals is essential.

Which non-animal alternatives did you consider for use in this project?

Although there are presently no non-animal models for behavioural responses, in vitro information e.g receptor binding/functional assays and chemical structural analysis will typically form part of the over profiling of candidate drugs.

Why were they not suitable?

Non-animal studies are not sufficient to fully evaluate behavioural responses and learned behaviours.
To fully assess the pharmacodynamic effects (effects of a drug on the body) of a new drug testing in animals is necessary. Only in a fully operational circulating system can the drug's distribution, metabolism, excretion which may alter or intensify the efficacy or adverse effects of the new medicine be fully understood.
For these reasons animal models remain essential in the development and safety assessment of new medicines.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The total estimated numbers of animals to be used have been determined based on previous projects over the past 10 years, anticipated future requirement, and in review of future services being developed and offered globally for regulatory non-clinical studies.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Studies will be designed under this licence such that the minimum number of animals will be used in order to obtain the maximum information, whilst the scientific objectives of each study are met, in accordance with regulatory requirements and agreed standard practices.
Data generated from pivotal studies will be statistically analysed, with comparisons drawn between control and treated groups, and reported in a format consistent with the ARRIVE guidelines published by the NC3Rs.

Where possible, studies will be designed in such a way that individual animals will be assessed for behaviour in a battery of tests as opposed to separate groups of animals for each test.
For studies within this project which are primarily designed for efficacy or investigative purposes, side effects may be detected which if considered unacceptable for the therapeutic area for which the drug is intended may lead to the cessation of its development early on. Having the opportunity to terminate the development of a drug as early as possible in the development process reduces the unnecessary use of animals.

Where possible, multiple behavioural assessments will be conducted in order to minimise the number of animals used and the need for separate standalone studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Our organisation has a wealth of experience and knowledge regarding regulatory requirements and the industry standards used, hence we are in an ideal situation to influence study designs when discussing requirements with our Sponsors and will always consider ways of reducing animal requirements. We also have available professionally trained statisticians to help design studies.
Study protocols are reviewed by the AWERB against known guidelines and the Company's ethical compliance policies. For regulatory studies, guidelines require the appropriate number of groups to clearly demonstrate the presence or absence of effects of the substance; core study designs are based on international guidelines where these exist. If not, use is made of literature and scientific principles of experimental design. Where appropriate, use is made of removing or limiting control groups and challenging the need for the use of both sexes. Additional justification will be requested from the study sponsor and reviewed by the PLH/AWERB for studies requiring a greater number of animals in order to better characterise the variability of a scientific endpoint.
Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies, with power-sample size calculations performed for specific studies if necessary to determine group size. Where group sizes allow, data are analysed statistically. However, due to the behavioural nature of studies within this project licence test agent effects are made by examination of data from each animal, rather than or in addition to simply assessing group mean values and statistical parameters.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The animals used during the course of this licence will be rodents. The models within this licence are behavior based and as such animals are required to be in a good state of health in order to perform tasks associated with some protocols.
In general, tests are conducted as follows;

- Tests which involve placing animals within a novel environment (e.g. maze or arena) and observing their natural behaviour within these environments for a relatively short period of time (e.g. 10 minutes).

- Tests which involve a 'task' such as shredding nesting material, burying marbles, pressing a lever, walking along a beam or rotating drum.

- Tests which require learning and memory recall, such as negotiating a maze for a positive reward e.g. radial arm maze, novel object recognition.

- Tests which require a reflex response to an external stimulus e.g. computerised auditory startle, pupil response to light, or response to an approaching object.

In all cases, the potential to cause stress or distress is minimal and short-lived. The assessments performed are not based on pain or fear, nor do they induce, or require a behavioural despair component.
Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies will be designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

Why can't you use animals that are less sentient?

Rodents are the animals with the lowest neurophysiological sensitivity that allows you to meet the aims of the studies. Scientific opinion, including that of the regulatory agencies, indicates the use of rodents within these study types are appropriate, and to use a non-rodent species should only be done if there is strong scientific justification to do so.
The tests within this licence are strongly behaviourally based and as such require the use of conscious animals .
Adult, juvenile or neonates may be used within the project licence.
Neonates will only be used for the purpose of inducing an autism spectrum disorder for which they will receive a single administration of valproic acid.

Juvenile animals may be required if certain aspects of behaviour or treatment are thought to be age sensitive e.g. exposure to cannabinoids may have greater effects in children and adolescents compare to adults.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
The severity limits specified are considered to be the minimum commensurate with achieving study objectives. Studies conducted under this licence are within the moderate severity category and it is expected that the majority of animals will have an actual severity that is the low end of moderate or mild. The actual severity is anticipated to be largely determined by procedural effects (such as repeat administration) rather than clinical signs resulting from administration of novel test agents.
All procedures are kept to the minimum commensurate with the study objective. Best practice guidelines for all animal care and use are followed.
It may be necessary to take serial blood samples from the study animals to monitor plasma levels of the test compound. The tail vein and jugular vein are accessible in rats and will provide adequate blood samples. Blood sampling from mice may be required on rare occasions. Where blood samples are required we will take them using the sampling site, volume, and frequency that has least welfare impact on the animals. Where possible samples will be taken under non-recovery anaesthesia. Microsampling (using very small volumes of blood, which causes less distress to an animal) will be utilized where methodology is available to do so.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Technical, scientific and regulatory developments will be monitored throughout the duration of this licence. Opportunities to introduce refinements will be evaluated by the Project Licence Holder and Animal Welfare Ethical Review Body to ensure the regulatory and scientific objectives of individual studies can be met whilst achieving the intended benefit. If required, validation studies will be conducted under this licence to ensure these objectives can be met.
Prior to the start of a particular programme or study, the client will be requested to provide information regarding known or potential effects/drug interactions that could be confounded by routine veterinary treatments or standard husbandry practices. Any such interactions are rare but with pre-planning, alternative treatments may be available (e.g. opioid vs NSAID analgesia, different bedding types etc).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Information issued by regulating authorities, the Home Office, cross company consortiums, in-house projects and scientific associations (e.g. the NC3Rs) will be reviewed and adopted as appropriate in

accordance with best practice.

Home Office

NON-TECHNICAL SUMMARY

21. Central Nervous System Determinants of the Transition from Acute to Chronic Pain

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Pain, Nociception, Central Nervous System, Fear

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Acute (normal) pain is a necessary sensory function that signals actual or potential damage, such as the heat of a fire. It is short lasting and is essential for survival, as it drives simple protective measures such as removing the hand from the fire. However, many people suffer from continuous, (chronic), painful conditions such as arthritis or back pain. In addition to the suffering incurred by the individual concerned, the knock on effects of suffering chronic pain often has detrimental effect on family, friends and society as a whole through loss of workforce and the cost of healthcare. For the majority of sufferers, the available drugs fail to provide

effective relief and often have unwanted side effects. Current treatments are largely ineffective and so new lines of research are needed to identify novel targets for the development of treatments.
Whilst the cause of pain (e.g. injury) is invariably remote from the brain, its perception is always at the level of consciousness within the brain itself. For most people pain stops after recovery from injury. However, in a significant proportion of individuals chronic pain persists beyond the period of tissue repair and can last for months or years. Why some individuals, and not others, are susceptible to this form of chronic pain is unknown. One mechanism of chronic pain might be due to the inability of the brain to forget a pain signal following injury, similar to the inability to forget a fearful memory in anxiety disorders (such as post traumatic stress). Testing an individuals ability to forget (extinguish) a fear memory might therefore predict how vulnerable they are to developing chronic pain.
The question that will be addressed in this project is how the original injury sets up changes in the brain that lead to a continued perception of pain at the level of consciousness.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Current medicines for the treatment of chronic pain are at best only partly effective and often have unwanted side effects. The outlined work aims to advance understanding of the mechanisms underlying the experience of pain, at the level of the brain, and thereby identify new strategies for the relief of pain. The results from these studies will provide important information relating to the way the brain regulates pain perception and why some individuals are more susceptible than others to chronic pain. In so doing we aim to identify new mechanisms that can be used to bring about the more effective relief of continuous pain for the benefit of people and animals.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The study will be carried out in rats because, like humans, they show variability in their susceptibility to chronic pain. Therefore, the information gained in rats will be directly relevant to development of pain-relieving strategies for humans and other animals. We will use an experimental design that minimises animal numbers while ensuring we achieve our scientific objectives. The study is expected to use 280 rats over five years.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

In order to investigate the susceptibility to chronic pain it will be necessary to evoke a pain state resulting from nerve injury that models human disease or injury. This will be achieved using techniques that the sensory experiences of animals brief escapable mechanical and thermal stimuli will be applied to the skin. In order to facilitate drug delivery or to record activity within the brain animals will undergo a small surgical procedure under general anaesthesia. Some animals will be maintained under terminal anaesthesia to minimise harm. In other animals, following surgery they will be given analgesics and are expected to recover uneventfully and return to normal behaviour with 24 hours. All animals will be humanely culled within 8 weeks at the end of the studies.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The neural circuits responsible for the perception of pain are located within the brain. The integrated neuronal circuitry involved cannot be adequately modelled by computer or using isolated cells cultures. Consequently, it is not possible to undertake these studies without using animals.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Affective and tests of nociception in animals are designed to assess emotional and physical behavioural responses to painful stimuli which is inevitably associated with distress and discomfort to the animal. Therefore, it is imperative to reduce the numbers of animals and use the minimum number possible to minimise pain and suffering.
In order to do this, for each of the proposed experiments, power calculations, based on data from published studies, will be used to determine the numbers of animals needed. Batches of animals will be tested and an assessment of the results will be carried out before experiments are continued, to ensure the minimum number of animals are used to make statistically significant conclusions.
Whenever possible experimenters will be blinded to treatment allocations and animals will be randomly assigned to study groups to ensure any findings are robust by minimising bias, meaning fewer animals should be needed for replication of results.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

A lot is already known about the functioning of the rat brain in relation to pain. Rats have been used extensively for similar studies in many laboratories worldwide because their brains share much in common with that of human and they response to pain is well characterised.
The behavioural responses to pain using outbred strains of rat have been shown to provide a good model for replicating the variation of human susceptibility to chronic pain. This choice of model, although more variable, should provide a better method to investigate our research questions and make results more robust and reliable. Results gained from experiments under this licence may provide further refinement for experimental rat models relating to chronic pain, and any such findings will be published and made available to the wider scientific community.
The models of pain induction and assessment selected for this work have been extensively validated and are least severe needed to undertake the study. In all cases the period of sensitisation will be kept to minimum required to obtain therapeutically meaningful results, while ensuring that observations made over time yield the maximum data from the minimum number of animals. Any refinement in methodology relating to pain induction and assessment that minimises suffering and distress, whilst maintaining meaningful results, will be made available to the wider scientific community.

Home Office

NON-TECHNICAL SUMMARY

22. Cerebrovascular health and function in ageing and Alzheimer’s disease

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Alzheimer's disease, Cerebrovasculature

Animal types	Life stages
Mice	adult, juvenile, embryo, neonate, pregnant, aged
Rats	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how risk factors for the development of Alzheimer's disease impact on the structure of the cerebrovasculature, how this affects clearance of solutes and fluids from the brain and the impact on brain function.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Alzheimer's disease (AD) affects more than 850,000 people in Britain and 35 million people worldwide. The annual cost of dementia is estimated at more than £30 billion/year in the UK alone. Current treatments for AD help to stabilise memory loss for a short period of time, but do not stop or reverse the disease. Risk factor for AD include old age, genetic factors and metabolic disease like obesity and diabetes. Women are also more likely to be affected than men. However, it is still not understood how these factors increase the risk of developing AD.

One of the hallmarks of AD is the build-up of beta-amyloid (Ab) in the brain. As it accumulates, Ab becomes toxic and kills brain cells, leading to dementia. In healthy individuals, Ab is cleared from the brain before it has a chance to accumulate. Blood vessels in the brain play a critical role in this process. They also help to form the blood-brain barrier, which is a physical barrier between the blood and the cells of the brain. This allows the brain to strictly regulate what gets into and out of the brain. Damage to blood vessels can slow down the removal of Ab from the brain and lead to its accumulation both in the brain and blood vessels themselves. This results in less blood flow to the brain and leakage of damaging components from the blood into the brain which contributes to the death of brain cells. Therefore, we believe that factors that increase the risk of developing AD do so in part by damaging the blood vessels of the brain, leading to increased amounts of Ab and decreased cognitive performance.

Results from this project will provide new information about how damage to blood vessels in the brain contributes the risk of developing AD and help to identify new ways in which AD may be prevented or treated.

What outputs do you think you will see at the end of this project?

This project will generate data from behavioural analyses and quantitative and qualitative observations represented in tables, graphs, databases and microscopy images. These data will be used to generate public lectures, posters and publications in scientific journals.

Who or what will benefit from these outputs, and how?

The findings from this project will give a better understanding of how factors such as age, diet and genotype affect the efficiency of Ab clearance from the brain. This will provide new information for the scientific and medical community as a whole and will highlight a new direction for effective treatments for AD to be developed. As such, these groups are likely to be the immediate beneficiaries of the results generated from the project. In the longer term, potential beneficiaries include health professionals who work with AD patients (e.g. gerontologist, psychologists), the commercial private sector (e.g. drug companies that develop AD-targeted

therapeutics), the general public and AD patients themselves.
How will you look to maximise the outputs of this work?

This data will be used by our group, our collaborators and other academic and clinical colleagues to whom the information will be disseminated at conferences and through publications. In addition, we will use our existing relationship with AD charities (e.g. Alzheimer's Research UK) to give public talks about our work within local communities.
Species and numbers of animals expected to be used

- Mice: 5700
- Rats: 1400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rodents are model organisms for choice as the least sentient mammal that can be reasonably used for these studies. There is a considerable body of data on the neurobiology of the rodent that is directly relevant to the functioning and pathology of the human brain. Further, genetically altered mice and rats replicate many aspects of human AD, including age-related pathology in the brain and cognitive impairment. Identification of factors that influence protein aggregation and solute clearance in animal models can be used to develop new therapies to treat human AD patients.
Typically, what will be done to an animal used in your project?

To understand how blood vessels contribute to clearance of solutes from the brain, animals will receive an injection of Ab or related proteins into the brains under anaesthesia. Some of these animals will have had a previous surgery to kill specific groups of brain cells that are known to die early in AD. A subset of animals will be treated with drugs or biological compounds that influence vessel function before or during the intracranial injection. Another group of animals will be treated with drugs that are designed to prevent the aggregation of Ab or other AD-related proteins to see if this treatment improves brain clearance pathways. The animals will be killed shortly afterwards with an overdose of anaesthesia and the pattern of distribution of Ab within the brain and blood vessels will be analysed. These experiments will be carried out in young, adult and aged wildtype animals and using GA animals that express mutations that mimic AD pathology or have abnormal blood vessels. To determine how modifiable risk factors for AD, such as obesity and diabetes affect vascular and cognitive function, wildtype and GA mice and rats will be fed either a normal or modified diet. This diet will be administered to male and/or females animals before, during and after pregnancy and/or lactation. A subset of foetal and neonatal offspring will be killed to determine the effects of parental diet on early brain and vascular development. At weaning, offspring with be fed either the same or opposite diet to that of the parents. A subset of animals will be used to assess fluid and solute movement in the brain using tracer injections as described above. Offspring will undergo behavioural analyses to test the effect of risk factors on behavioural parameters that are affected in AD. In some cases, the same animal will be tested at different times during its lifespan to determine how behaviour changes with age. A subset of animals will be treated with drugs that target metabolic disease or will be given a running wheel for voluntary exercise before or during the behavioural tests. At the end of the experiment, animals will be killed with an overdose of anaesthesia and their brains will be analysed to determine the effect of diet and dietary interventions on brain and vessel structure. These experiments will be carried out in foetal, neonatal, young, adult and aged wildtype and GA animals to assess changes across the lifespan.

What are the expected impacts and/or adverse effects for the animals during your project?

For experiments requiring surgery, there is a low risk that animals may develop hypothermia during surgery and post-operatively. During the post-operative period (e.g. ~1 week), there is a low risk that animals may experience pain or infection. Feeding a modified diet high in fat or low in protein will likely result in sustained weight gain or loss. High fat-fed animals may also develop obesity-related health issues. For experiments using behavioural tests, there is a low risk that animals will be stressed by the novel environment during behavioural tasks (~60-90 mins) and of fighting injuries of male mice when they are re-homed together after testing. Some GA models may develop behavioural abnormalities (e.g. hyperactivity).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

None of the procedures in this project will exceed a moderate severity. Most animals (~90%) used in breeding protocols will be transferred to an experimental protocol before the onset of a phenotype. A minority of breeders (~10%) that are kept up to 12 months may experience moderate severity as a consequence of a progressive phenotype. All animals on experimental protocols will experience a moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The complex nature of the mammalian brain and blood vessels makes it difficult to study movement of fluid and solutes into and out of the brain using animal-free mechanisms. Rodents are model organisms for choice as the least sentient mammal that can be reasonably used for these studies because there is a lot of information about the brain and behaviour of rodents that is directly relevant to humans. Further, genetically altered mice and rats replicate many aspects of human AD, including pathological and behavioural changes which can be used to develop new treatments.

Which non-animal alternatives did you consider for use in this project?

Tissue culture models of the blood-brain barrier and computer models of fluid movement within the brain.

Why were they not suitable?

Where possible, we will use tissue culture and computer models to test basic functions of cerebral blood vessels and to model the dynamics of fluid movement under normal and diseased conditions. However, neither of these models fully replicate the complexity of the cerebrovasculature or the properties of fluid exchange between the brain and blood. In addition, complex behavioural and cognitive functions that mimic human behaviour cannot be fully assessed in non-mammalian systems.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may

include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers have been calculated using power calculations that are based on effect sizes generated either from preliminary or previous experiments or as an estimate where no pre-existing data exists. Each outcome has been assessed individually and the animal numbers in each study group represents the minimum required to detect statistical significance of 5% between the experimental groups.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where possible, calculations have been made based on data collected from preliminary or previous experiments (generated either by our group or in the published literature) to ensure that effect sizes are accurate. The NC3Rs Experimental Design Assistant is also used when formulating experimental design. We have also consulted with statisticians to ensure that power calculations and statistical tests used for data analysis are appropriate.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For heterozygous GA strains, breeding protocols will be used to minimise the number of offspring without the target gene and wildtype littermates will be used as controls where possible. For experiments where animals are aged, all major organs will be collected from the animals at the end of the experiment and banked either for future use or shared with colleagues.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project will use wildtype and GA mice and rats. Protocols for rodent husbandry and health management are well established and there are many rodent-based resources that can be consulted for standard operating procedures. In addition, the size of these animals also makes it ideal for physiological manipulations. Rodents have high reproductive capacity and a relatively short generation time that facilitates multigenerational studies and studies on the consequences of ageing.

Why can’t you use animals that are less sentient?

Where appropriate, foetal animals will be used to assess the effects of risk factors on brain and vascular structure. Similarly, acute intracerebral injections and tissue collection will be carried out in terminally anaesthetised animals. Recovery surgeries and ageing protocols will only be used where there is a need to study time-dependent effects or to evaluate a phenotype across the lifespan.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For procedures that require surgery, levels of anaesthesia will be maintained at sufficient depth for the animal to feel no pain. Aseptic techniques and sterile instruments will be used to help prevent postoperative infection. The animals' body temperature will be maintained throughout surgery and during recovery. Animals will be administered analgesic pre- and post-operatively and given additional doses if they show signs of pain or discomfort. Animals will be monitored during recovery until they regain consciousness and daily for the first post-operative week for signs of illness (e.g. loss of body weight, dehydration, poor grooming, social isolation) or infection.

For procedures that require dietary manipulation, including food restriction, animals will be weighed daily until a stable weight is achieved and then a minimum of 5 days/week to ensure that body weight is maintained at target weight. Animals will be given additional food immediately if they approach their target weight and dominant animals will be separated where animals are housed together.

During behavioural tests, animals will be closely monitored and removed immediately if they show signs of illness or distress. Animals will be handled appropriately throughout the procedures and where possible habituated to the novel environment of the behavioural task. Animals will be monitored daily for fighting injuries or signs of illness and distress following re-housing in groups.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Consultation with PREPARE and ARRIVE guidelines and use of NC3Rs and related resource hubs, including published literature.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Experimental design will be done in consultation with published literature as well as PREPARE and ARRIVE guidelines. NC3Rs and related resource hubs will be routinely checked for current standard operating protocols and advice will be sought from the NVS before undertaking new procedures.

Home Office

NON-TECHNICAL SUMMARY

23. Characterizing the function of a gene family involved in stress sensing in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

heart, skeletal muscle, cyclic nucleotides, regeneration, disease

Animal types	Life stages
Zebra fish	adult, embryo, neonate, juvenile

Retrospective assessment
The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits
Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

A gene family involved in stress sensing is important for preserving the structure and function of the heart and skeletal muscle. We are characterizing zebrafishes carrying altered (mutant) forms of these genes to define their role in health and disease.
A retrospective assessment of these aims will be due by 02 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A gene family involved in stress sensing encode proteins that bind a signalling molecule produced in cells in response to hormonal (for example adrenaline) stimulation. It is vital for the heart, as it regulates the speed and force of contraction and is also required in skeletal muscle to preserve its structure and function. Patients suffering from cardiac and/or skeletal muscle disease have been identified to carry mutations in one of the three members of this gene family. Introducing these mutations in zebrafish and studying the resulting heart and skeletal muscle defects will help to characterize the activities of these proteins and to define their function in health and disease. Recently, we began to explore the function of this family of proteins in cardiac and skeletal muscle regeneration. Defining the reasons for impaired heart and muscle regeneration in zebrafish mutants will lead to a deeper understanding of the roles of these proteins and possibly may lead to a novel class of drugs, which might be able to improve healing after an infarct.

What outputs do you think you will see at the end of this project?

- This work is expected to provide new information about the role of these stress-sensing genes in cardiac and skeletal muscle regeneration and will help to elucidate the mechanisms of how they are controlling pathways that regulate the response of muscle cells to stress.
- The primary expected benefit is the publication of new scientific knowledge about how these stress sensors control cardiac and skeletal muscle regeneration.
- New information will also be obtained about the mechanisms how mutations in these genes are causing heart and skeletal muscle disease.
- The results of this work will be presented at scientific meetings and subsequently in publications in peer-reviewed journals to ensure wide dissemination to the appropriate audience.
- Research may in longer term also result in novel therapeutic products as these stress sensing proteins represent a unique molecular target, which is involved in many vital functions of the heart and skeletal muscle.

Who or what will benefit from these outputs, and how?

The scientific research community will benefit from this work. Primary recipients are the research communities working on cyclic nucleotide signalling, which includes approximately 500 researchers worldwide. Another research community that will be interested are researchers that work on cardiac regeneration in zebrafish with an estimated number of about 100 researchers worldwide. Our work will also be of interest to the wider heart and skeletal muscle research communities, which are estimated to be approximately 10,000 and 2,000 researchers, respectively. In the short term we will provide novel insight into the mechanisms of how these proteins are mediating stress signalling in the heart. In the longer term, the research may yield novel pathways, which could be exploited for the development of novel drugs.
The work on cardiac regeneration in zebrafish may help to develop novel therapies to alter the fate of myocardial infarction, which currently is a highly detrimental disease-causing heart failure and death. Understanding how these genes are involved in heart and muscle regeneration in the short term will lead to novel information about the cellular pathways involved in this process. It is uncertain whether this novel knowledge will indeed have an impact on clinical practise and alter the outcome of myocardial infarction. It may have a 20% chance that the new knowledge generated might be of benefit in the clinics in the longer term.

How will you look to maximise the outputs of this work?

The results of this work will be presented at scientific meetings, will be published in peer-reviewed journals to ensure wide dissemination to the appropriate audience. We also regularly writing review articles to further foster dissemination to the various research communities that are interested in our work. These information routes will encourage further collaborations and potentially lead to translational opportunities.

Species and numbers of animals expected to be used

- Zebra fish: 8,100 adults

Predicted harms
Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The gene family involved in stress sensing consists of three genes, which are present in all vertebrates including zebrafish. The high conservation grade allows to model mutations in zebrafish, which have been discovered in patients. The adult zebrafish has the ability to fully regenerate the heart after injury. In mammals this ability is also present at birth but is lost one week after birth. Overall zebrafish and mammals display very similar processes in response to heart injury. However, while the zebrafish is able to heal its heart, the mammalian heart will substitute cardiac muscle with scar tissue.
The scar tissue will not participate in pumping blood, which in the long run might cause heart failure. The adult zebrafish may serve as a model to unravel mechanisms involved in cardiac regeneration that lie dormant in the adult mammalian heart but potentially could be reactivated. Studying heart regeneration in adult zebrafish lacking one member of the gene family involved in stress sensing therefore may help to identify novel pathways that are involved in this process, which when activated help to recover the human heart from myocardial infarction. We are also interested in skeletal muscle regeneration and membrane healing. Skeletal muscle unlike the heart maintains the ability to regenerate as muscle contains a specialised cell population, the so-called satellite cells that are able to reconstitute muscle after injury. Skeletal muscle injury is commonly present after sport or walking downhill for example. Restoration of muscle structure is impaired in animals lacking these stress

sensing genes and we are interested to identify the reason for this failure. Knowledge gained in this way may help to understand the function of these proteins in muscle regeneration and this knowledge might help to treat patients carrying mutations in one of these genes. Patients carrying point mutations in these genes also develop an irregular heartbeat as do zebrafishes carrying mutations found in patients. Characterising the underlying reasons for irregular heartbeat remains challenging but it is hoped that the study of adult zebrafish hearts in this context will help to elucidate the underlying pathology. For this purpose, fishes will be subjected to electrocardiography in order to assess the presence of an irregular heartbeat. Since these genes are involved in stress-signalling, the heart abnormalities will become fully apparent only after stress induction. This is accomplished by injecting substances which trigger a faster heartbeat and it is in this situation that the mutant heart is expected to show its abnormal response to stress.

Typically, what will be done to an animal used in your project?

Adult zebrafishes are anaesthetised and placed ventral side up on a damp sponge. A small incision is made through the chest to access the heart. A small part of the ventricular wall is freeze-injured by applying a cryoprobe precooled in liquid nitrogen. Fishes are returned to water and after overnight recovery from surgery the fishes are taken back to the aquarium facility. At 1-365 days after injury, euthanasia is performed by an overdose of anaesthetics.
For skeletal muscle injury, the tail musculature of the anesthetized fish will either be injected with a small volume of a toxin, cryoinjured with a cold probe, or wounded by a needle. These treatments are causing skeletal muscle injury. The healing response will be studied usually in the next four weeks and not longer than 6 months after surgery. Animals will be euthanised at the end of the experiment.
These proteins are important regulators in stress signalling, which in the heart lead among other responses in a faster heart rate. In case these stress-sensing genes are not functioning properly the response to stress is causing an abnormal heart beating. We want to elucidate the underlying cellular and molecular causes of this abnormal stress response. Mutants will be subjected to electrocardiography (ECG) analysis at baseline and after injecting substances, which trigger a molecular stress response. Under these circumstances, the mutant heart will display an abnormal heart beating pattern, which will help us to define the underlying molecular mechanisms. ECG analysis is a non-invasive method. After anaesthesia the animals will be placed ventral side up on a damp sponge and the ECG electrodes will be lowered on the body surface of the fish in order to pick up the electrical signals. Substances will be injected into the abdominal cavity at the appropriate volume and dose and subsequently returned to continue ECG analysis. The procedure will be repeated up to five times in order to study changes in clinical phenotype as a function of age of the animal. Animals will be euthanised at the end of the experiment.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals that do not recover from cryoinjury normally die in the first 24 hr after surgery. Usually, the mortality of the procedure is around 10%. Successfully operated animals do not display signs of discomfort and are swimming and feeding normally. The animals will be studied usually up to 90 days after injury and in some cases up to one year after surgery.

Animals subjected to muscle injury will show no mortality and only some minor discomfort due to the muscle injury, which however will improve as regeneration will take place. The animals will be studied usually up to 4 weeks after injury and in some cases up to six months after surgery.
Animals subjected to ECG analysis are expected not to show any mortality and the procedures will only transiently cause an abnormal heart beating, which will return to nearly normal heart beating at the end of the procedure.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

- Heart lesioning is a severe procedure and 10% are expected to experience this severity level while 90% will have a moderate outcome.
- Muscle lesioning is a moderate procedure and 100% of the animals are experiencing this severity level.
- ECG phenotyping is a mild procedure and 100% of the animals are expected to show this severity level.

What will happen to animals at the end of this project?

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 02 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our aim is to model heart and muscle disease found in patients carrying mutations in genes involved in stress sensing. These diseases are complex and are only partially modelled using cell culture models. Moreover, we want to study heart and muscle regeneration. Also in this case, cell cultures only can model certain aspects of the wounding response Regeneration of heart and skeletal muscle involves many different cell types. These processes therefore too complex to be investigated in cell culture. While the work in animals will be complemented with experiments in cultured cells, a complete substitution of animal work is currently not possible.

Which non-animal alternatives did you consider for use in this project?

We also work with primary cultured cardiac myocytes, frog oocytes and other cell lines. Some of our work involves preparations of the whole heart or part of it, which are maintained in a viable and functional condition outside of the body for several hours, which also helps to limit the number animals used for our research.

Why were they not suitable?

Work in isolated cardiac muscle cells, frog oocytes or cell lines are suitable for certain aspects of our research. However, these cell models do not show the full spectrum of responses that we see in the intact animal and therefore are not sufficient to fully substitute the work in animals.

A retrospective assessment of replacement will be due by 02 May 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The animal numbers are estimated based on the demand of the previous license.
Protocol 1 Obtaining Zebrafish Gametes (mild procedure): We estimate the need for approximately 100 adult fishes for five years.
Protocol 2 Generation of Founders (F0 Generation) (mild procedure): We estimate the need for approximately 500 adult fishes per 5 years. The production of transgenic animals is a routine procedure. However, depending on the genetic manipulation several attempts might be needed before the right genetic alteration is observed. For the generation of single mutations sometimes only 1% or less of the injected oocytes contain the correct genetic alteration.
Protocol 3 Breeding and Maintenance of Genetically Altered Zebrafish (mild procedure): The number of fishes required for the maintenance of the various lines (breeding as well as the crossings to generate homozygous animals or crossing in any reporter gene or transgenes) will amount to 5,000 adult fishes for five years.
Protocol 4 Heart Lesioning (severe procedure): We estimate a total of 1,500 adult fishes for five years to allow for a detailed analysis of the regeneration defect in the mutants. For some of the examinations, the small size of the fish heart will require the use of several animals per time point to obtain sufficient amount of tissue.

Protocol 5 Skeletal Muscle Lesioning (moderate procedure): We estimate a total of 500 adult fishes for five years

Protocol 6 Phenotyping (ECG) mild procedure: We estimate a total of 500 adult fishes for five years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

During the experimental design phase, we will utilize online tools such as the NC3R Experimental Design Assistant in order to reduce the number of animals used in this project. We minimise live animal use by capturing as much ex vivo and in vitro data as possible from each animal. A large part of the experimental work in my group is carried out ex vivo. The PREPARE guidelines will be utilised to optimally plan a new set of experiments and to avoid any duplication of efforts. The ARRIVE guidelines will be followed and in particular the ARRIVAL Essential 10 in order to standardise the reporting of animal research and improve the reproducibility of the research outcome.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We continuously trying to optimise the breeding and genotyping of the animals. We will also do pilot studies if a new experimental protocol will be implemented. In order to estimate the required numbers of animals to find significant differences between experimental and control group. If possible, tissue will be used for different experiments
A retrospective assessment of reduction will be due by 02 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement
Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Zebrafish is an established model for biomedical research and the genetics and working protocols are very well developed. We breed the least number of animals for line maintenance and for the various experimental protocols. Injury triggers heart regeneration in zebrafish and the molecular response is very similar to those observed in higher vertebrates including the human heart. Cryoinjury is a less invasive procedure than surgical resection, which is causing bleeding and therefore may cause a higher mortality. Importantly, cryoinjury triggers transient scar formation and therefore models the situation after myocardial infarction in the human heart quite well.

Why can’t you use animals that are less sentient?

For the purpose of studying gene function, most of the scientific research we are planning requires the use of adult stages. This is for example true to model heart and muscle disease in patients or to study the role of these genes in cardiac regeneration. While regeneration could in principle be also studied in the immature larval stages, at this developmental stage many of the cell types present in the adult heart are not yet present. Observations made at this stage will therefore not be directly applicable to the adult heart. Similarly, patients develop muscle and heart disease in response to mutations only as young adults at the earliest, some of the mutations trigger a late onset of the disease and only appear when patients are in their forties or even older. Therefore, it is mandatory to study the role of these genes in the adult zebrafish.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have noted and incorporated a number of refinements to each protocol since we started to use zebrafishes more than 15 years ago. We continue to monitor animals closely, and with NVS constantly assess possible improvements in post-operative care, pain management, and animal environments.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow the latest guidance of publications by the zebrafish community such as: Aleström P, D'Angelo L, Midtlyng PJ, et al. Zebrafish: Housing and husbandry recommendations. Lab Anim. 2019;23677219869037. doi:10.1177/0023677219869037. We follow PREPARE guidelines, plan and conduct studies according to the ARRIVE guidelines and use NC3R guidelines to ensure our animal experiments are as robust and reproducible as possible.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We are regularly attending scientific meetings and talk to colleagues using the zebrafish model. We read the monthly updates from the NC3Rs on their events and publications and their e-Learning resources all of which detail advances in 3Rs technologies and best practice and provide information how to put these in place.
A retrospective assessment of refinement will be due by 02 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

24. Circadian regulation of pulmonary immunity

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Lung, Circadian, Inflammation, Immunity

Animal types	Life stages
Mice	juvenile, adult, neonate, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how the circadian clock interacts with the immune system to regulate how the lung responds when faced with a challenge via a biological agent that causes disease or illness.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Pulmonary diseases such as asthma and chronic obstructive pulmonary disease (COPD) affect 1 in 5 people in the UK, and prior to the outbreak of COVID-19 in 2020, lung diseases were responsible for more than 700,000 hospital admissions in the UK each year (data from the British Lung Foundation). The symptoms of chronic pulmonary inflammatory diseases, often show daily variation in their occurrence, worsening during the night. Similarly, biomarkers used in the clinics to assess disease severity fluctuate over the course of the 24h day. The circadian clock is a timing mechanism which synchronises animal physiology to the 24h environment created by the earth rotating on its axis. This biological timer regulates numerous aspects of physiology, including sleep wake cycles, feeding and metabolism, hormone secretion and the immune system. Disruption of the circadian clock is associated with increased prevalence of inflammatory diseases, including pulmonary disorders.

The circadian clock plays a critical role in regulating the normal working of the immune system and ensuring appropriate inflammatory responses are mounted when the system is challenged. This work investigates mechanistic links between the biological timing and immune systems to understand the involvement of the clock in regulating pulmonary physiology. An ultimate goal of these studies is to reveal novel therapeutic targets or improve existing therapeutic regimes to treat pulmonary disease through the use of biological timing (chronotherapy).

What outputs do you think you will see at the end of this project?

A major output from this project will be an advance in our knowledge regarding circadian control of immune responses within the pulmonary system. More specifically, information generated from these studies will contribute to our understanding of how the circadian timing system affects the development and progression of human pulmonary diseases such as chronic obstructive pulmonary disease (COPD), asthma and pulmonary fibrosis. This information will further our understanding of how circadian disruption (a consequence of rotating shift-work) may impact on lung function in both health and disease. Additionally it is predicted that data generated by studies outlined here will have a positive impact on the diagnosis and treatment of human pulmonary disease. An example here is the implementation of chronotherapy (for example timing therapeutic interventions with the peak of disease symptoms) to treat asthma.

In order to maximise these positive benefits, it is essential that information obtained from studies outlined here is disseminated effectively. This will be achieved through: publication in academic journals (reaching basic scientists and clinicians); presentation of data at seminars and conferences (targeted at clinicians and basic scientists); and through public engagement events.

A secondary benefit of this programme of work is the advancement of research methods. This may be in the form of refining or enhancing current methods or through the development of new transgenic mouse lines. Furthermore, the data generated from this project will be made available to other researchers in the scientific community at the earliest appropriate time therefore informing further scientific discovery within our research community.

Who or what will benefit from these outputs, and how?

Healthcare sector: Outputs from this research will be of benefit to the healthcare sector. There is a growing

understanding of the importance of considering circadian time in the diagnosis and treatment of pulmonary disease. This work will further develop our knowledge of how the circadian timing system interacts with processes underlying human pulmonary conditions. It is hoped that these studies will promote further incorporation of "clock logic" into clinical practice in the long-term. That may be through standardising the time of day at which a patient's blood or sputum is sampled for a disease biomarker, or through recommending the best time of day at which to take medication.
Scientific community: This programme of work will generate new research tools and advance research methods which will be shared with the scientific community (through publications and seminars) in order to benefit scientific discovery worldwide longer term. Although the focus of this project is pulmonary disease, pathologies and processes underlying these diseases are applicable more broadly in the field of immunology. Thus data generated in this project will advance our basic understanding of clock control of immunity and will be of benefit to the wider research community. Data will be disseminated at the earliest opportunity benefitting the scientific community as soon as possible.
General public: The importance of the circadian clock and good sleep hygiene for maintenance of health is becoming widely recognised by the general public. Information obtained through this project will be of interest to the general public, including people engaging in shift-work and patients suffering with pulmonary disease. It is becoming more and more evident the disruption of the circadian clock has negative consequences on health. Whilst sometimes circadian disruption cannot be avoided (e.g. shift workers), for some individuals small lifestyle changes may have a positive impact on health. In order to engage the general public we will continue to reach out via public engagement events run by the University and charities.

How will you look to maximise the outputs of this work?

Outputs from this work will be published in highly visible journals targeting a multi-disciplinary audience. The research group has an excellent track record of publishing data in high impact journals read by basic scientists and clinicians. Furthermore, through the use of social media (Twitter) and the establishment press office, we will publicise these publications as widely as possible in order to engage with the general public. In the past, this has led to opportunities to present our research on the radio, television and news websites. We appreciate that it is important to disseminate negative findings to minimise replication of experiments across research institutes. In addition to publication of articles, on going work will be presented at international and national meetings aimed at circadian biologists, immunologists and respiratory clinicians.
To maximise the benefit of our research we will continue to engage with the general public and relevant patient groups through organised events. These activities are important for maintaining public interest in our research and also further developing trust in UK research.

Species and numbers of animals expected to be used

- Mice: 24 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

These studies will utilise juvenile and adult mice, which may be genetically modified. Mice are the most appropriate species for these studies as the systems which we are studying here (the body clock and immune

system) are well reproduced between mice and humans.

Typically, what will be done to an animal used in your project?

Animals may be monitored non-invasively for behaviour under normal or altered environmental conditions (typically for periods lasting 2-6 weeks). These environmental manipulations include changes in the light/dark cycle, changes to the composition of the diet, or changes in timing of food availability. More rarely, animals may undergo physiological monitoring using implanted telemetry devices (requiring a brief surgical procedure) or imaging facilities (under recovery anaesthesia). Rarely repeated imaging may be utilised (up to 8 times in one day). Changes to rhythmic biological signals (such as glucocorticoid hormones, melatonin hormones or microbial metabolites) may be instigated through implantation of hormone pellets under the skin (brief surgical procedure) or through application of antibiotics.
Approximately one third of the animals utilised in this project will undergo a procedure to induce pulmonary inflammation. To instigate pulmonary inflammation one of four approaches will be utilised, each modelling a different human chronic inflammatory disorder. (1) Inflammatory mediators (e.g. lipopolysaccharide - a component of the bacterial cell wall) may be applied acutely (20 minutes) via an aerosol to directly target the lung. (2) Allergic inflammation may be induced via application of a reagent that the animal has previously been sensitised to, such as house dust mite. (3) Fibrosis of the lungs may be induced through brief local application of reagents that cause local lung injury (bleomycin) resulting in tissue remodelling in the longer term (2-4 weeks). In rare instances animals which have developed fibrosis will receive a second inflammatory insult of aerosolised lipopolysaccharide (a bacterial product). (4) Animals may undergo a surgical procedure (under terminal anaesthesia) where the lungs are mechanically ventilated for a period of time. Prior to, or during these four procedures the immune system may be manipulated, this includes the application of reagents to target specific pathways (e.g. through the use of antibodies). On rare occasions the immune system may be manipulated further through irradiation to deplete host haematopoietic cells before replacement with donor cells. Additionally, reagents may be administered which target the clock or the immune system to establish their effects on inflammatory processes.
Animals utilised in these models of lung inflammation may be assessed using *in vivo* imaging, collection of small volume blood samples and/or assessment of respiratory mechanics (under terminal anaesthesia).
In addition to these procedures, mice will be used in this project for breeding, and for provision of cells and tissues for *ex vivo* studies.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals may experience mild adverse effects such as temporary stress or brief pain and discomfort. Temporary stress may be induced by a brief period of restraint (e.g. in order to administer an injection) or alterations in their housing environment (e.g. single housing or alteration of the light cycle). Mice may experience a brief period of pain and discomfort in response to dosing (e.g. injection or intra-nasal dosing) or blood sampling, or following surgical intervention to implant a telemetry device or hormone pellet. In these instances the stress and discomfort will be transient.
Weight loss may occur after manipulation of the diet (either through altered timing of food availability or altered composition). Weight loss will be transient and will either reverse and return to the starting level as the animal fully adjusts to a new regime (e.g. food available only during the daytime) or may stabilise below the starting level if the total amount of food available is reduced. Furthermore, administration of antibiotics in the drinking water and irradiation protocols may results in transient weight loss.
Acute models of pulmonary inflammation are associated with transient discomfort and stress due to application of inflammatory reagents and may result in brief periods of mild respiratory distress. The chronic models of pulmonary inflammation (allergic inflammation and fibrosis) may also be associated with longer periods of mild respiratory distress (hours), transient weight loss and occasionally loss of condition.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The studies outlined in this project will result in a cumulative impact to the animals that are subthreshold to mild (approximately 60 percent) or moderate (approximately 40 percent) severity rating.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In the context of organ-level inflammation, it is currently not yet possible to replicate the complex multicell environment underpinning disease. Thus, *in vitro* cellular models and *in silico* models have limited application as a replacement for studies of pulmonary inflammation. In order to study complex interactions between the circadian timing system and immune responses occurring in the lung, mouse models are the most appropriate approach.

Which non-animal alternatives did you consider for use in this project?

In vitro approaches include the use of single-type cell lines or co-culture systems (where two or more relevant cell types are studied together). We utilise these methods to inform the direction of animal studies. *In vitro* assays can be used to test how genetic or pharmacological interventions alter the function of the cell intrinsic clock and/or regulation of inflammatory processes. For example, *in vitro* assays using cell lines or primary cells allow us to first test a diverse range of potential therapeutic interventions and identify a small number of candidate molecules with the most potential for subsequent use *in vivo*. However these approaches cannot replicate the complex environment of the lung and cannot replace the use of animals.

Why were they not suitable?

We have successfully utilised cell lines (such as Human Bronchial Epithelial Cells) to examine circadian control of inflammatory responses. Furthermore, on going projects in the laboratory involve exploring the potential of various cell lines in monoculture or co-culture to help address our research questions. However, it is critical that any cell lines utilised are circadian rhythmic. Our experience here however, is that cultured cell lines are often non-rhythmic, in contrast to the situation *in vivo* where they may be rhythmic. This may be an artefact of multiple passages of cell lines, or due to the absence of other signals (hormones or cytokines) that these cells would normally be exposed to *in vivo*. Thus their use is somewhat limited. Furthermore, *in vitro* assays cannot adequately model the complete array of inflammatory responses or address how systemic timing signals may modify these responses.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been

taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our grouping has extensive experience with the methodologies and approaches outlined here and of running projects of a similar scope. Consequently, estimates of animal numbers are based firstly on previous experience with the models to be utilised and the types of data generated, and secondly with careful consideration of the experimental design.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Throughout the duration of this licence, we will carefully consider the design of each experiment. As the project develops, and we build up further data sets, we will utilise this information to further refine the experimental design. When considering the design of experiments underpinning this project we have consulted with statisticians to gain specialist advice on the types of experiments that will be undertaken and the nature of the datasets that will be collected. We will continue to do this as the work develops. We will be utilising purpose written software (such as the NC3Rs Experimental Design Assistant) to further support experimental planning and randomisation and blinding.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Throughout this project we will continue to strive to optimise animal use wherever possible. This starts with efficient breeding of transgenic mouse lines, achieved through close monitoring of colonies which is facilitated by the use of specialist colony management software and effective communication between our laboratories and technical staff managing the colonies. We aim to minimise numbers of animals bred whilst still achieving adequately powered, age and sex-matched groups of experimental animals. When individual projects have been completed and data is being prepared for publication, breeding of relevant mouse strains will be minimised until a suitable time to preserve the colony by freezing down gametes.
Pilot studies are utilised to optimise experimental conditions when we are developing new approaches. Where it is appropriate we utilise technologies that permit longitudinal assessments in the same animal. Furthermore, we always look to maximise the amount of data that we can gather from a single sample using the latest technologies to their full capacity (for example using mass cytometry to assess expression of numerous proteins in one individual cell).
At the end of each experiment we carefully consider which tissues to collect with future studies in mind. By building a well archived tissue bank we are able to utilise existing samples in the laboratory to test new protocols or reagents (e.g. new antibodies) without the need to utilise further animals. We also make our banked tissue available to our collaborators.
Through providing our collaborators and the wider scientific community with access to data generated through our studies (through depositing large datasets in online data repositories) we aim to maximise the scientific knowledge than can be obtained from our animal studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

A substantial proportion of the methods that we will use in the project are non-invasive and involve environmental manipulations to study effects on circadian physiology and/or immunity. This includes manipulation of the lighting, meal composition or meal timing. These processes themselves are unlikely to cause suffering or distress. On occasion these manipulations may require animals to be single housed, to minimise potential distress in this situation, where possible we will supply mice with environmental enrichment (e.g. plastic tubes, plastic igloos, wooden logs or nestlets). Some animals may be subject to a brief surgical procedure to implant a telemetry device or hormone pellet. We have significantly refined our approach to glucocorticoid hormone replacement by establishing in previous studies that it is not necessary to surgically remove the adrenal glands (the major source of these hormones) prior to pellet implantation. This has reduced requirements for surgical intervention and improved recovery times.
Where we seek to administer reagents to experimental animals we utilise the most refined route of administration possible. For example, we routinely administer antibiotics in the drinking water rather than through oral gavage. Furthermore we always seek to minimise the numbers of doses of a treatment in order to achieve our objective, this may involve pilot studies to identify an optimal dosing regime.
This project involves the use of models of pulmonary inflammation. We chose to use the most refined models available to address our experimental objectives. For example in order to establish a local acute inflammation within the lung, we utilise aerosolised administration of lipopolysaccharide (a component of the bacterial cell wall) rather than dosing with bacteria itself (e.g. Streptococcus pneumoniae, which induces a more profound inflammation which can rapidly spread to the periphery). Other models we utilise include administration of a chemical to the lungs which causes local tissue damage and scaring, which has been optimised in terms of dosing route, dosage and time post administration in order to induce a robust and consistent response whilst causing the least harm and suffering to experimental animals. Models of allergic inflammation utilised in this licence are relatively mild with the period of lung inflammation an animal experiences being minimised as much as possible whilst not compromising experimental aims. Further, assessment of respiratory mechanics in these models is performed utilising *in vivo* approaches which generates the most robust and reliable data and is performed under terminal anaesthesia.

Why can't you use animals that are less sentient?

We cannot replace these studies in mice with studies in another species (such as insects or fish) to achieve our objectives, as they lack the complex immune and circadian systems seen in higher order species. Whilst we are able to carry out some of our studies in terminally anesthetised animals (for example lung ventilation studies) this is not appropriate for the majority of our pulmonary inflammatory models as they take a prolonged period of time to initiate disease and/or we require longer term assessment of disease progression.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We routinely seek to minimise stress and discomfort to animals during our work and achieve this by ensuring all researchers utilise appropriate animal handling techniques (e.g. tube handling to remove animals from their cage) and by using environmental enrichment wherever possible, especially in instances where it is necessary to singly house an animal.
Occasionally animals may undergo a brief surgical procedure (e.g. to implant a telemetry device or hormone pellet). After surgery, animals are closely monitored and post-operative care provided (pain management with analgesics and provision of extra fluids). Analgesics may be provided in the form of a palatable gel to encourage voluntary ingestion.
Animals which develop allergic pulmonary inflammation or pulmonary fibrosis are monitored regularly, including monitoring weight loss and general condition. Where transient weight loss is expected as a consequence of this condition, soft food (wet mash) will be provided on the cage floor as well as environmental enrichment.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure that our experiments are conducted in the most refined ways we continually assess our experimental design and re-assess approaches if the opportunity arises. We stay informed about best practice guidelines by referring to information provided by Laboratory Animal Science Association (LASA) and NC3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our group stays informed about recent advances in 3R approaches by staying up to date with NC3Rs recommendations and developments. This information from the NC3Rs is obtained through interaction with their website, local seminars, contact with their staff and through social media (Twitter). We also discuss further refinement opportunities with our NVS and NACWO and through interaction with colleagues at conferences, workshops and seminars.

Home Office

NON-TECHNICAL SUMMARY

25. Complement in chronic kidney diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

proteinuria, kidney failure, complement, fibrosis

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The finding of protein in the urine (proteinuria) identifies a group of patients at risk of kidney failure. These patients are most likely to develop progressive kidney disease and to need dialysis, but there have been no new treatments for over 20 years. Leakage of protein into the urine in kidney diseases causes damage to cells lining the kidney tubes ultimately resulting in scarring and loss of normal kidney function. Proteinuria is damages the kidney by activating in the kidney an element of the patients' own immune system - known as the complement system. Targeting the complement system is therefore an attractive option to treat kidney disease. There are

several new genetic models in mice and complement inhibiting agents that could be used to explore this potential therapeutic avenue.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This project is therefore of direct relevance to human disease. Currently there have been no significant advances in treatments for chronic kidney disease for 20 years. Increased knowledge of the underlying mechanisms of kidney diseases has shown how proteinuria is of key importance, and new understanding of the pathological pathways activated in the kidney under these circumstances has provided new opportunities. Complement has been shown to be of key importance in kidney diseases. At the same time new modifiers of the complement pathways have become available as potential therapies. Therefore resting these potential therapies in these models is timely and likely to be very beneficial. A positive outcome will lead to direct evidence to support the design of new clinical trials in patients with proteinuria and kidney disease. These findings may be very generalisable to patients with a wide range of kidney diseases and will reduce the numbers requiring dialysis or transplantation. Furthermore new markers of disease and response to treatment will be identified.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The project will use mice – either normal wild type mice or mice genetically manipulated to modify their complement system.
The project may require up to 1750 animals.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Animals may be subjected to the removal of one kidney or the obstruction of a ureter at surgery, and other less invasive procedures. These are of moderate or lower severity. Repeated injections of ip saline or BSA solutions have been reported to cause pulmonary oedema. This is mitigated by minimising the volumes injected, and the protocols are based on the literature and our own previous experience. Trauma from repeated injections is minimised by varying injection sites. Adriamycin can be associated with systemic toxicity and death in mice if given at too high doses. This will be avoided by minimising doses administered according to previous experience and the published literature. Previous experience shows us that these procedures are generally well tolerated since we have previously performed similar studies have refined the techniques.
Animals will receive analgesia and close monitoring for distress. Humane endpoints will be determined according to daily observations recorded and scored using score charts (see embedded). These observations will focus on parameters based on body weight, coat condition, body functions, and behaviour, with each parameter assigned a pre-determined severity score. It is anticipated that the great majority of animals will not meet these endpoints and will reach the planned end of the experimental protocols.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The objective is to study mechanisms by which complement participates in progression of kidney diseases. While cell culture models have allowed valuable progress to be made, these are imperfect to model of the behaviour of whole kidneys which comprise multiple different cell types. Many kidney cells in culture also lose many of their specialised functions and do not properly resemble the same cells in a whole kidney. In view of this complexity, non-animal techniques, such as mathematical modelling, are unable to reproduce the necessary complexity.
For studies of inflammation, interactions between kidney cells and infiltrating cells, such as white blood cells, that occur during inflammation cannot be modelled without knowing the cell populations involved, which can only be determined in animal models.

Reduction

Explain how you will assure the use of minimum numbers of animals.

To minimise animal numbers we draw heavily on our experience with these models and the numbers in groups needed to obtain significant results. Power calculations have also been used derived from the published scientific literature.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice will be used for these studies to allow us to exploit the local availability of genetically altered mouse strains which will enable us to test more effectively the underlying mechanisms of complement involvement in kidney disease.
The study proposes to use the most refined versions of proteinuria or ureteric obstruction currently available in mice. Unnecessary animal suffering will be further minimised by drawing on our previous experience with these experiments and by refining our techniques where necessary using pilot studies with small numbers per experimental group.
Mice are the most relevant species to use and have previously been used successfully to generate rodent models of renal disease relevant to human disease.

Home Office

NON-TECHNICAL SUMMARY

26.Consequences and complications associated with diabetes

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Diabetes, Hypoglycaemia, Hyperglycaemia, Complications

Animal types	Life stages
Mice	juvenile, adult, neonate, pregnant, embryo
Rats	juvenile, adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

This project will investigate the impact of diabetes, and in particular how fluctuations in blood sugar levels (glycaemic variability; GV) affect organ systems such as the brain and heart. By examining the effects of glucose dysregulation on organ function and/or integrity we hope to identify the underlying mechanisms that may lead to diabetes-associated complications.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The incidence of diabetes is increasing exponentially and with the age of the population rising the incidence of diabetes-associated complications will be a huge financial and societal burden. Improving our understanding of the mechanisms underlying these complications may improve our treatment strategies and minimise the long-term consequences.

What outputs do you think you will see at the end of this project?

The immediate outputs of this project will be scientific publications in high quality, peer-reviewed, high impact journals appropriate to the field of research, communication to relevant national & international scientific conferences and press releases disseminating the research findings.

Who or what will benefit from these outputs, and how?

The findings of this research will, in the short term, improve our understanding of factors that may contribute to complications associated with diabetes. For example, if we identify that oxidative stress plays a pivotal role in the development of the cognitive dysfunction associated with diabetes, we will look at measures to reduce this such as antioxidant supplementation. These findings will be disseminated to the wider community through interaction with our clinical collaborators, publication in peer-reviewed journals and presentation at local, National and International meetings. In the long term, the findings of these studies will be translated into the clinic. By working closely with our clinical colleagues, we hope to ensure that our animal models recapitulate the human condition as much as possible.

How will you look to maximise the outputs of this work?

The output of this work will be published in peer-reviewed journals and presented at National and International conferences. We will continue to work in close collaboration with clinicians and with other collaborators within the scientific field. A paper is in preparation to describe in detail the mouse model of Type 1 diabetes that we have developed and techniques that we have optimised (e.g blood sampling from the tail vein without restraint) to disseminate this knowledge to the wider scientific community. The publication of negative data is crucial to minimise unnecessary experiments being performed and this will take place via platforms such as BMC Research Notes.

Species and numbers of animals expected to be used

- Mice: 1500
- Rats: 600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use adult rodents (mouse and rat) in this project as they are widely used in this field of research and share a similar physiological and behavioural response to hypoglycaemia as do humans i.e. polydipsia and polyuria at the onset of diabetes. The use of transgenic models enables us to examine the impact of individual genes on diabetes progression or response to hypoglycaemia. The complications associated with diabetes usually occur over a period of several years in humans and therefore we will study adult rodents in this project.

Typically, what will be done to an animal used in your project?

The animals (rat and mouse) used in this project will be made diabetic by the injection of a compound that preferentially destroys the insulin-secreting cells of the pancreas (beta-cells). We will then give the animals insulin replacement therapy to maintain health, in a very similar manner to insulin therapy given to individuals with Type 1 diabetes or long-duration type 2 diabetes. To mimic the hypoglycaemic episodes experienced by those on insulin therapy the animals will be given insulin injections and blood glucose allowed to fall to the hypoglycaemic range (<3.0mml/l). We know that this will lead to a suppression of the normal counterregulatory response to hypoglycaemia. We will then assess the impact that diabetes and/or hypoglycaemia have on aspects of cardiovascular function (Objective 2), cognition (Option 3), mechanisms that may be able to restore the ability to respond to hypoglycaemia (Objective 4) or how ischaemic preconditioning may alter the cardiovascular system in diabetes. These experiments will typically last 2-3 months, and animals will undergo no more than 8 procedures.

What are the expected impacts and/or adverse effects for the animals during your project?

We anticipate transient pain following injection of STZ however, this is minimised by using Hanks Balanced Salt Solution (HBSS) rather than citric acid that is normally used. The animals may be lethargic following STZ administration, but this is transient with normal movement and eating behaviour returning within an hour. Weight loss may occur in the week following T1D induction and confirmation of hyperglycaemia, but this is restored following implantation of the insulin pellet and better glycaemic control. Insulin-induced hypoglycaemia may cause transient discomfort at the site of injection and care will be taken to alternate the site of injection. The animals will typically become slow and lethargic during hypoglycaemia, initially, they may display foraging behaviour as glucose drops. Animals will typically become hypoglycaemic within 30 mins of insulin injection and will be monitored closely to minimise the chance of severe hypoglycaemia. If glucose continues to fall food will be provided and if the animal is unable to stand on all 4 paws dextrose will be administered to restore euglycaemia. This procedure will last no more than 2 hours (max 1 hour at hypoglycaemia). All animals undergoing surgical procedures will be given appropriate analgesia to minimise pain. Weight loss is likely to occur in the first 2 days following surgery, but soft food will be given to minimise weight loss. Guidance will be sought from the NVS and NACWO should any animal display signs of abnormal behaviour or any unexpected change in physical appearance.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Adverse effects depend on the studies being performed and mainly relate to surgery or the development of diabetes (approximately 60% of our studies), very much as we see in humans. Diabetes, as in human subjects is monitored by checking blood sugar levels and treated with insulin when needed. Surgical procedures such as

the insertion of a catheter into an artery or vein are of moderate severity and performed under general anaesthesia, this will account for <20% of animals on this licence. Pain killers are used routinely, and antibiotics as required. When animals have completed all the *in vivo* studies they are humanely euthanized.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Diabetes is a complex disease characterised by elevated blood sugar (glucose) levels and alterations in insulin production (Type 1 diabetes) or sensitivity (Type 2 diabetes). Although we can model some aspects of the condition *in vitro* for example the exposure of cultured neurons to high levels of glucose, this does fully replicate the interaction between different cell types (neurons, astrocytes, glial cells) or tissues that have a fundamental role in the development of many of the co-morbidities associated with diabetes. Replicating the complex and variable conditions that occur in a disease state such as diabetes is extremely difficult and limits the applicability of these models to disease.

Which non-animal alternatives did you consider for use in this project?

Where possible we perform studies in cell systems first to ensure that a candidate gene or pathway is important to the things we want to study. Subsequently, highly specialised techniques have been developed for measuring the animal's response to challenges such as low or high glucose and this means we can compare much smaller groups of animals. Techniques have been developed that allow us to study the animal while awake so we can conduct repeated tests on the same animal rather than using lots of groups.

Why were they not suitable?

Replicating the complex and variable conditions that occur in a disease state such as diabetes *in vitro* is extremely difficult due to the complex interaction between cell types and organ systems (pancreas, brain, liver, muscle, adipose tissue) and this limits the applicability of these models to disease.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals required for this project has been calculated based on previous experience using these models We have also estimated the number of pollutants and complex mixtures available for our research and the experiments required to understand their toxicity mechanisms.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experimental design will be in line with ARRIVE guidelines and will follow NC3R guidance using the Experimental Design Assistant. Studies will be kept as simple as practically possible to maximise the information obtained from the minimum number of animals. Advice will be sought as necessary from statistical sources, locally or online, concerning the minimum number of experimental animals required to allow a sufficiently powerful statistical analysis.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use highly specialised techniques that have been developed for measuring the animal's response to challenges such as low or high glucose and this means we can compare much smaller groups of animals. Techniques have been developed that allow us to study the animal while awake so we can conduct repeated tests on the same animal rather than using lots of groups.

Vascular imaging will be performed in the same animal at several time points to establish a profile of disease progression/development, therefore, reducing the overall number of animals required.

In all cases, tissues will be collected and archived for subsequent analysis by both our group and others working in the field of diabetes.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse and rat models that we propose to use in this project are well-studied models used frequently in metabolic research which helps when comparing results to other groups. As sugar is a critical fuel for the brain for the rodent as it is for the human, we find that rodents like humans become fat and develop diabetes when given too much food. Also, they respond in the same way to both single and multiple episodes of low glucose as do humans with type 1 diabetes and so they are a good animal model to study.

We minimize welfare costs by using highly trained staff to conduct all our studies who have now many years' experience in working with animals and using the techniques we employ. New staff are rigorously trained. All studies are carried out following recommended guidelines under the guidance of the local veterinary team and in a facility with highly trained staff.

Why can't you use animals that are less sentient?

Diabetes is a complex disease and complications associated with diabetes develop over time. Terminal anaesthesia alters the hormonal and biochemical response to hypoglycaemia and therefore does not replicate the human condition.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Prior to any experimental procedures animals will be handled for a minimum of 1 week to acclimate the animal to the user and vice versa. Whilst on a study, animals will be weighed and body condition assessed weekly by PIL and/or trained staff at the animal resource unit. Following surgery postoperative care and pain management will be provided following advice from the NVS. We will use a refined method of diabetes induction through STZ injection by using Hanks Balanced Salt Solution
(HBSS; pH 7) rather than citric acid (pH 4.2) which is used as the standard vehicle for STZ administration. Likewise, recurrent hypoglycaemia will be performed in the animals home cage following a minimum of 2 weeks handling with blood samples from the freely moving animals from the tail vein. This is a well established procedure in the lab and we have demonstrated causes minimal suffering to the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Best practice guidance for experiments will be acquired from LASA, NC3R and other appropriate websites.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I will regularly visit the NC3Rs website and others recommended by the local named information officer.

Home Office

NON-TECHNICAL SUMMARY

27. Control of C. difficile Infection

Project duration

5 years 0 months

Project purpose

- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult
Hamsters	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our long-term aim is to develop a solution to the control of *C. difficile* infection. In the first instance this would be a prophylactic solution that prevents or reduces susceptibility to infection.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

C. difficile is a disease afflicting humans worldwide and in the UK almost 28,000 people are infected each year with over 1,000 deaths. Indeed, it is the predominant, antibiotic-induced hospital-acquired infection that is found in industrialised countries. This disease mostly occurs in the elderly and for those in hospital so it is important that this disease is controlled whether this be by vaccination or by the use of other interventions. No vaccine is currently available while existing treatment options are not 100% effective and often entail the use of antibiotics for which there is a globally recognised need to find alternatives.

What outputs do you think you will see at the end of this project?

This project should lead to significant improvements in our understanding of *C. difficile* infection, an important hospital-acquired infection that leads to more than 1,000 deaths annually. This work will be disseminated to the scientific community through publications and, it is hoped, to the wider community through press-releases etc. Most importantly, our work will support the development of new treatments for this disease either to prevent infection or for therapy. After 5-years, if not sooner, it is possible that we are ready to enter evaluation in humans.

Who or what will benefit from these outputs, and how?

This project should lead to the introduction of our prototype product into clinical evaluation and we anticipate applying for human studies (phase 1) within the tenure of this project.

How will you look to maximise the outputs of this work?

We would like to publicise our results and raise awareness. This would occur through publications, conferences and participation with advocacy groups with whom we sponsor.

Species and numbers of animals expected to be used

- Mice: 4,500
- Hamsters: 1,300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice and hamsters will be used to evaluate the ability of vaccines or other novel interventions to prevent C. difficile infection. The scientific community working on this disease numbers several hundred groups worldwide

and all use mice and hamsters to evaluate the efficacy of emerging treatments. It is important that data generated here can be compared to related work that has been published. This is an important and critical step in developing a treatment for an unmet clinical need.

Typically, what will be done to an animal used in your project?

Animals will be given test vaccines or biological interventions by the oral route. Controls may include animals injected with the same or similar substance for comparison. Animals will then be given a course of antibiotics (in drinking water or by oral dosing) after which they will be administered an infective dose of C. difficile. This will be given orally and sufficient to cause infection. Animals will typically be housed in groups for 4-8 weeks but during the period of infection they will be housed individually in cages. In all cases animals are re-housed to 1 animal/cage 12-24h before the first administration of antibiotic in Protocols 2 and 3. Two types of infection will be assessed, a) colonisation, that is the ability of the pathogen (C. difficile) to grow and survive within the animal and b) virulence, which is the manifestation of symptoms of disease in the animal.

What are the expected impacts and/or adverse effects for the animals during your project?

For animals where colonisation is assessed animals will experience no symptoms nor discomfort. For animals where the virulence of disease is to be assessed the animals will experience some level of discomfort including lethargy, lack of appetite and mild diarrhoea. The period of this discomfort is likely to be at most 12h.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Protocol 1: 100% of animals the severity is MILD
Protocol 2: Mice - 70% of infected animals will show symptoms (MODERATE) unless protection is observed. For hamsters, 100% of infected animals will show symptoms (MODERATE) unless protected.
Protocol 3: 100% of animals the severity is MILD
Protocol 4: Mice - 70% of infected animals will show symptoms (MODERATE) unless protection is observed. For hamsters, 100% of infected animals will show symptoms (MODERATE) unless protected.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

With current scientific product development a biologic with prophylactic or therapeutic properties (that is to be assessed in humans) it is required that efficacy is first demonstrated in one and preferably two animal models should they exist. In the case of C. difficile infection both mice and hamsters provide determination of whether a treatment option can prevent infection. Use of both animal systems is recognised by the worldwide scientific community as is evident from the plethora of international publications relating to this disease. Accordingly, regulators who facilitate evaluation of a biologic in humans will demand evidence that a product demonstrates efficacy using established animal models of infection.

Which non-animal alternatives did you consider for use in this project?

Currently, there are no non-animal alternatives available. It is worth emphasising that the decision to enter animal testing is itself determined by a number of in vitro tests that taken together support evaluation in animals. While in vitro tests are not confirmatory of efficacy they play an important role in the decision to evaluate in animals and the likelihood of success.

Why were they not suitable?

A disease caused by a bacterium is nearly always multi-factorial, that is, the manifestations of disease result from a number of independent events. For example, production of toxins, attachment to a substrate, induction of innate immunity etc. Each of these events can be assessed individually using in vitro tests and taken together provide an indication that a test product may work but only in an animal model can this be categorically defined as positive or negative.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Previous studies will have used power calculations to determine group sizes and the validity of these calculations demonstrated from previous studies. As a rule, numbers of animals used per group should be sufficient to enable statistical significance between groups. Since the studies contained here have the same primary objectives we can use the same group sizes. If the primary objective will be different a new power calculation will be performed to determine animal numbers.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In the long-term animal numbers are reduced by careful project design reducing the need for further experiments. A core tenant of the design of animal experiments is the correct use of statistics (for interpretation of significance) and power calculations (to determine group size). The level of variability in some primary endpoints necessitates larger numbers to achieve statistically and biologically meaningful data. The most appropriate statistical tests Mann-Whitney (or the students t-test) for significance and ANOVA for analysis of variance between groups.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In our experience for studies are to be undertaken where large number of animals are to be used then small pilot studies using 2-3 animals/group are preferable. This is particularly important where a challenge dose must be determined or a dosing regimen established. Although statistical significance is not determined, in the long term, this avoids a negative outcome involving large numbers of animals.

Additional factors are to ensure the use of the same animal supplier (where possible) and the use of similar aged animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice and hamsters (Golden Syrian) will be used to evaluate C. difficile infection using two basic approaches:
a) Asymptomatic colonisation. This model uses mice only and shows no symptoms of disease.

This model is informative for understanding whether a test product prevents proliferation of the pathogen (C. difficile) in the GI-tract. It can also provide data on the presence or absence of toxins.
b) Virulence models. In mice and hamsters using these models of infection animals will develop symptoms of disease. Using an accurate assessment of symptoms animals confirmed to be infected are killed before more acute symptoms develop ensuring minimal discomfort to the infected animal/s. To ensure accurate assessment frequent and then continuous monitoring of animals is undertaken and only using trained staff entirely familiar with the infection process and its associated symptoms in animals.

In general, vaccines and biological interventions are always tested first in mice using the asymptomatic models. Demonstration of efficacy in these models is then followed by evaluation in the asymptomatic models (mice preferred) if required and deemed necessary.
The least invasive route of substance administration and using flexible gavage needles will be used where possible. Negative control groups will be minimised whenever statistically feasible.

Why can’t you use animals that are less sentient?

Mice and hamsters are used since these are the lowest vertebrate group that can be used for in vivo evaluation of C. difficile interventions or vaccines.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Improvements in any process can always be made and we are receptive to any suggestions as well as our own assessments. We are keen to consider and if possible implement non-aversive handling of animals as well as practices for enrichment of quality of life for animals housed under solitary conditions. We will implement these where possible using only suitably trained staff and discussion with the host facility as necessary.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

NC3R guidelines. We will follow the NC3Rs guidelines on the "Responsibility in the use of animals in bioscience research" and consult all the relevant references listed therein.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By interaction with the Facility Director. Continuous monitoring of publications and the NC3Rs website for new and alternative models that could be implemented as part of this project and relevant information is circulated by

AWERB. Whenever possible we will implement these refinements into our studies.

Home Office

NON-TECHNICAL SUMMARY

28. Decision Making From Synapses To Circuits

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Decision making, value calculation, hippocampus, physiology, behaviour

Animal types	Life stages
Mice	adult, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We are working to understand how neurons in the brain communicate with each other to allow them to encode

emotional behaviours and make decisions.
A retrospective assessment of these aims will be due by 24 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Problems with the communication between neurons in the brain underlie the vast majority of neurodegenerative and neuropsychiatric diseases, and so our aim is to find novel ways to combat these diseases by gaining a greater understanding of the processes that they destroy.

What outputs do you think you will see at the end of this project?

The project comprises basic scientific research that will increase our understanding of how the brain supports decision-making behaviour. The outputs of this project will be new scientific findings, describing how neural activity in the hippocampus (and connected brain regions) supports value- and memory-based decision-making. The outputs will primarily take the form of publications in peer reviewed journals, but will also be disseminated at academic conferences and to the lay public via popular science initiatives. Materials, data and methods may, where appropriate, be disseminated online.

Who or what will benefit from these outputs, and how?

In the short timescale, the primary beneficiaries will be other scientists working in the field of learning and decision-making research. Our research will inform our fundamental understanding of how neural networks work in healthy adults, and how this is altered by experience during adolescence.
In the medium term, the basic science knowledge gained will likely inform broader fields of scientific enquiry, some of which have the potential for clinical translation. For example, understanding how memory networks malfunction after adolescent social isolation may inform strategies for developing new pharmaceutical or therapeutic interventions.
In the long-term, a detailed understanding of the neural-network level mechanisms for learning and memory will be an invaluable aid to designing interventions for mental health disorders of all types. For example, the neural circuit changes that promote the transition to mental illness are increasingly viewed to be extremely specific: effecting only specific cell types and the connections between them. Our research aims to uncover these specific alterations, and how they relate to specific behavioural phenotypes and symptoms. We hope that this will provide new druggable targets that are both potent and specific, and will allow for better, more personalised treatment, and the minimisation of off-target side effects.

How will you look to maximise the outputs of this work?

We will disseminate our research by publishing results in peer reviewed journals. We aim to publish all results, including those that do not confirm our hypotheses.
We will also present our work to academic peers at scientific conferences (national and international), and engage with the popular science media in order to disseminate our results to the general public.
Raw data and analysis methods will be shared with the scientific community (following peer-reviewed publication), to allow other groups to gain insights from our experiments, and reduce replication of work.

Species and numbers of animals expected to be used

- Mice: 7500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will use adult mice. We know a lot about the anatomy and physiology of the mouse brain, and in particular of the parts of the brain to be studied in this project. Mice also offer unmatched access to genetic tools, allowing us to use genetic techniques to record from and manipulate functionally/genetically/anatomically defined ensembles of cells. Together with the ability of mice to rapidly and flexibly carry out value and memory guided decisions, this means that we are well-placed to fill in substantial missing section of our knowledge: how patterns of neural activity influences decisionmaking.

Typically, what will be done to an animal used in your project?

In the most typical experiment, mice will undergo a surgical procedure under general anaesthesia, to carry out chronic (long-term) attachment of devices for monitoring neural activity to the skull of the animal. Analgesia will be provided during the surgery and during recovery. Following recovery, the attached devices do not, in themselves, cause any pain or distress to the animal.
Animals will then undergo experiments in which neural activity is monitored simultaneously with behavioural testing. Mice will learn to play a 'game' where there are correct and incorrect answers - mice will be rewarded with sugar water when it chooses the correct answer.
Animals will typically be motivated to learn using appetitive (desirable) rewards such as sweet liquids such as strawberry milkshake, and minimal levels of water restriction. In a smaller number of experiments, mice may also learn using negative reinforcers such as air-puff to the face or mild static shock, or from psychostimulant rewards such as cocaine or amphetamine.
Neural recording and behavioural testing experiments typically continue for weeks, or even possibly months.
At the end of the experiment, animals will be euthanised, using an overdose of an anaesthetic agent.

What are the expected impacts and/or adverse effects for the animals during your project?

Some animals may feel pain or discomfort during the recovery from surgery (1-2 days). To mitigate this, analgesia will be given to the animal.
Some animals may experience more than average weight loss following food or water restriction. In these cases, animals will immediately be removed from the experiment and provided with freely accessible food and/or water.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

30% of animals will experience the severity category 'Mild'.
70% of animals will experience the severity category 'Moderate'.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 24 March 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

During this project we will investigate how the brain is connected to carry out specific functions, and how this wiring is altered by experience. Crucially we will also monitor how these circuits encode and respond to the environment during complex decision-making behaviours. In order to investigate this problem we have to study these neuronal circuits in animals as the animals carry out these behaviours.

Which non-animal alternatives did you consider for use in this project?

Computational modelling, in vitro cell culture, human research, research on less sentient animals (e.g. fruit flies / nematode worms)

Why were they not suitable?

There are no computer models or equivalent that can accurately and effectively model these phenomena, and so experiments on living tissue are required. The data obtained during this project will allow for more accurate and precise modelling in the future.
No cultured cell lines are available to study the mechanisms that control synaptic connectivity, and so acute tissue must be used.
Although implantation of chronic electrodes is possible in humans, it is only permissible in a small numbers of clinical situations, and therefore not practical to answer the questions proposed. While increasingly human brain slices are becoming feasible, again these are strictly limited, and most often from diseased tissue. In addition, the specificity required to understand the functioning of these circuits is beyond current human-based techniques.
Less sentient species do not have the homologous circuitry to the mammalian decision-making circuitry investigated in this proposal.

A retrospective assessment of replacement will be due by 24 March 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of experimental animals have been estimated using a) power analyses, b) experience from previous published studies of effect sizes, and group sizes necessary to test effects.
A pure power analysis approach is not always appropriate for in vivo neural recording experiments, as the number of animals required will depend on the success rate of neural recording (numbers of neurons per animal). It is therefore necessary also use estimates based on previous experience of similar experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will seek advice from the University's applied statistics advisors.
We will follow the ARRIVE and PREPARE guidelines and use the NC3Rs Experimental Design Assistant.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies, allowing us to explore whether hypothesised experimental effects may be present, before committing larger number of animals.
Technical developments which enable us to monitor the activities of larger numbers of brain cells in each animal.
Use of computational models that enable us to make highly specific testable predictions about the role of hippocampus and other structures during behaviour, minimising the number of experiments required to reach a conclusion.
Most procedures involve long-term experimentation with the same animals, which significantly reduces the number of animals needed to reach statistically significant conclusions.

A retrospective assessment of reduction will be due by 24 March 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The model animal used will be mice.
The major methods used will be:
Stereotaxic surgery. This is necessary in order to inject and implant substances to identify, record from and manipulate defined neuronal circuitry both in vitro and in vivo. Appropriate anaesthetic/analgesic regimens will be used to minimise pain. e.g. delivery of pre-operative analgesia, as discussed with the NVS and Animal Facility Staff.
Maximum injection / infusion parameters will be strictly adhered to. In the very unlikely case that this is not possible due to extenuating circumstances, any changes will be discussed with the NVS.
Behavioural training. This is necessary to assess cognitive capabilities in animals. The large majority of these tests will use only appetitive (rewarding) stimuli, hence the only harm is mild food or water deprivation.
In vivo neural manipulation and recording. This is necessary in order to be able to draw direct functional links between neural activity and behaviour. Neural recording implants do not cause suffering and distress in themselves, hence the potential for pain and suffering is confined to the post-surgical period (in which analgesia will be provided, see below for details).

Why can't you use animals that are less sentient?

Mice will be used for this project as they represent the least sentient species appropriate for this type of work.

Decades of research has also resulted in highly advanced and efficient techniques developed for the mouse as opposed to other species. For example, there are excellent stereotaxic maps of the mouse brain, allowing accurate targeting of injections to specific brain regions. Coupled with my expertise in stereotaxic surgery, this results in very high success rate in our experiments (greater than 80% success rate in targeting of brain regions).
The mouse is also high genetically tractable, allowing transgenic identification of specific cell types crucial to the fulfilment of the project
.
Finally, as the proposal aims to investigate how neurons are utilised during behaviour, terminally anaesthetised mice would not be appropriate.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Almost all of our behavioural tests will use appetitive (rewarding) stimuli, the only aversive tests used will involve, for example, bright lights/loud noises or mild air puffs. Animals will be motivated to seek reward using mild food (~20% of restricted animals) or water (~80% of restricted animals) deprivation. We will only use the minimum levels of deprivation necessary to achieve uniform consistent behavioural results.
Appropriate anaesthetic/analgesic regimens will be used to minimise pain. e.g. delivery of preoperative analgesia, as discussed with the NVS and Animal Facility Staff.

Maximum injection / infusion parameters will be strictly adhered to. In the very unlikely case that this is not possible due to extenuating circumstances, any changes will be discussed with the NVS.
Housing cages will be spacious and enriched with e.g. rodent toys, chewable materials such as wood, running wheels, shelter, unless these interfere with the experimental design. In addition, where possible animals will be group housed post surgery using strategies devised in collaboration with our local NVS and NACWO.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Local NVS and NACWO
Resources hosted on the NC3Rs website, in particular:
- ARRIVE guidelines on experimental design and reporting results.

- 'Procedures with Care': 'Aseptic Technique in Rodent Surgery'.

- Rodent housing and husbandry

- Rat and Mouse Grimace scales

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep in constant contact with local NVS and NACWO to ensure we are maintaining best practice.
We will liaise with in-house NC3Rs representative to ensure we are up to date with current best practice.
We will use the resources published on the NC3Rs website to ensure that the group undergoes continuous training and professional development with respect to the 3Rs.
We will follow technological advances in the published scientific literature, allowing more efficient recording techniques (yielding more neuronal data per animal), miniaturising recording equipment (leading to a refined animal experience) or allowing recording in more naturalistic settings (for example, wireless recording).

A retrospective assessment of refinement will be due by 24 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

29. Defining the role of G protein coupled receptors in the brain and the therapeutic potential of targeting these receptors in neurological disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Alzheimer's disease, G protein coupled receptors, Schizophrenia, Pharmacology, Neurological behaviours

Animal types	Life stages
Mice	juvenile, adult, neonate
Rats	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project licence is aimed at continuing studies on the role of G protein coupled receptors (GPCRs) in the regulation of the nervous system including brain and neuronal function and how we might target this class of cell surface receptors in the treatment of neurological disease. The GPCR family consist of many hundreds of different receptors and are the most successful drug targets known to man. Despite this the full potential of the GPCR family has yet to be realised. The reason for this is that we lack fundamental understanding of the biology of many of these receptors and how best to target them in disease. In this licence we will conduct experiments focused on the biology and therapeutic potential of GPCRs in the nervous system.
A retrospective assessment of these aims will be due by 20 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

GPCRs are involved in key neuronal processes such as learning and memory and that selective stimulation of certain types of GPCRs can relieve symptoms and slow progression of neurological diseases including neurodegenerative disease. Hence studying them can lead to better understanding of how the brain works and the design of drugs that can impact on diseases such as Alzheimer's disease.

What outputs do you think you will see at the end of this project?

In the neuroscience project we aim to establish the role of GPCRs in the regulation of neurological responses such as learning and memory, anxiety-like behaviours and locomotion. We also expect to determine the impact of GPCRs particularly the muscarinic receptor family in the symptoms and progression of neurodegenerative disease, schizophrenia and possibly other neurological diseases.
These discoveries will be disseminated in the following ways;

1. Peer review literature

2. Scientific meetings in the form of talks and poster presentations

3. To the general public in the form of press releases, public seminars and social media

We also expect these discoveries to result in further grant applications and both charitable and government grants.
In addition, we have the anticipation that these studies would change the direction of drug company research opening up new clinical trials and impact on drug discovery strategy.

Who or what will benefit from these outputs, and how?

Academic Community - will benefit from an understanding of fundamental biology of GPCRs and the understanding of the best ways to target GPCRs to regulate pathophysiological responses.
Pharmaceutical/drug discovery community – will benefit from the validation of new GPCR targets in human disease and an appreciation of the pharmacological principles that can be applied to drug design.
General public – will benefit from the prospect that new methods will be developed to apply to drug discovery against some of the currently most intractable diseases including Alzheimer's disease.

How will you look to maximise the outputs of this work?

In terms of publications in the scientific literature and presenting in research meetings we are very experienced in these areas with strong relationships with editors of the top journals as well as being well connected with organisers of major research meetings. Hence we anticipate that we will continue to have strong outputs through these routes. We have also been developing our outputs via social media with both institutional and personal social media outlets being developed. Finally we are improving our public out-reach with visits to local prisons, schools and presenting to politicians where we present our research and discuss animal research ethics.

Species and numbers of animals expected to be used

- Mice: 20,000
- Rats: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The overarching purpose of this protocol is to gain greater understanding of the role and therapeutic potential of GPCRs, or GPCR-regulated pathways, in later-stage neurodegenerative disease. The aim is to determine if by activating (or deactivating) GPCRs, or GPCR-regulated pathways, we can alter the electrical brain activity and biochemical processes in the brain to influence progression of neurodegenerative disease.
As such we are planning to test rodents models because we and others have established that these models closely resemble human systems and we can mimic human neurological disease. Hence by determining the activity of our drugs/substances and effects of our genetic modifications in rodent systems we are able to determine the role and therapeutic value of GPCRs. We can best do this in adult animals but we can also investigate how our receptors work by conducting studies on primary neuronal cultures derived from new born animals. Hence this study is best suited to adult and new born animals.

Typically, what will be done to an animal used in your project?

1. Animals (mainly mice but occasionally rats) will be inoculated with brain preparations made from animals or humans that have had neuroidegenerative disease e.g. brain preparation from prion diseased mice or brain preparations from humans that have died from Alzheimer's disease.

2. Animals (e.g. mice or rats that are either genetically modified and/or have neurodegenerative disease) can then undergo behavioural or neurological testing and/or live animal imaging and/or blood sampling and/or monitoring of body temperature and locomotor activity and/or monitoring electrical activity and brain signalling. These steps can be conducted both with or without administration of drugs/substances, that are either delivered minutes/hours before biochemical/electrical/behavioural testings (i.e. acutely) or days before testing (i.e. chronically).

3. The experience described in point 2 above will also be that of genetically modified mice expressing variant genes coding for GPCR receptor proteins and signalling pathways activated by GPCRs.

What are the expected impacts and/or adverse effects for the animals during your project?

Mice are inoculated with misfolded protein such as prions/tau/alpha synuclein in order to develop progressive and terminal neurodegenerative disease. Although some of the animals in this protocol will be humanely killed before they reach late stages of disease a proportion of the animals (approximately 8%) will be maintained until late stages of disease, where they will likely develop physical symptoms, we propose a severe category. It is anticipated that some of the mice that enter late-stage disease may die before they arrive at a humane end point.
Furthermore, from our previous experience when testing for adverse responses to GPCR drugs approximately 2% of animals develop physical symptoms that can include pain, weight loss, seizures, or abnormal behaviour.

Other than this the drug/substances administration, behavioural, imaging, surgical and drug administration procedures described here will fall into mild category as described in the above protocol expect for an occasional (approximately 1%) a moderate.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately 8% of mice/rats will be maintained until late stages of disease, where they will likely develop physical symptoms that result in a severe category.
Approximately 2% of animals develop physical symptoms associated with drug/substance administration that result in a severe category.
All other animals will fall into a mild category with occasional (approximately 5%) moderate .

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 20 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and

why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are using a number of sophisticated approaches to mimic brain responses thereby offering the opportunity to replace the use of animals in neuroscience research. This includes working with optic physicists to develop an "optical brain" that mimics brain activity using light and chemists who are similarly developing a "chemical brain" that responds to chemicals in the same way as a brain. However, currently none of these approaches come close to accurately mimicking brain activity. Hence to investigate the three primary areas of this project namely the processes that regulate brain activity via a group of proteins called G protein coupled receptors (GPCRs), ii) how we might change brain activity through drugs that act on GPCRs and iii) how we might cure the symptoms, and slow the progress, of neurodegenerative disease - we need to conduct experiments on animals.

Which non-animal alternatives did you consider for use in this project?

We will be using human brain tissue (both normal and disease) obtained from registered human tissue banks for the neuroscience projects described here.

Why were they not suitable?

Human tissue is preferable to mouse tissue however it is not possible to employ genetics to validate the receptor targets in human tissue. It is also not possible to trial drugs that target our receptors in humans - rather we can only test the response to our drug treatment in resected tissue or from post mortem samples. Hence we aim to combine the animal studies with human tissue studies to probe the function of GPCRs in human disease. The ultimate aim will be to subsequently develop drugs based on our findings to trial in human clinical studies.
A retrospective assessment of replacement will be due by 20 May 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have a great deal of experience in many of the behavioural and physiological experiments that the mice will be used in and have also a great deal of experience in the breeding and maintenance of our lines. Based on previous experience we have been able to apply power calculations in collaboration with statisticians. This has allowed us to estimate how many animals we need for the experiments described in this protocol.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We are constantly making attempts to reduce the number of animals in the following ways;

1. By conducting biochemical experiments, such as determining the changes in the proteins in the brain, we are looking for early markers of neurological disease that can give reliable indications of drug efficacy thereby reducing the number of animals and the time they are exposed to disease. This is particularly the case in the neurodegeneration studies but also in our inflammatory models.

2. We are using human tissue with increasing frequency to reduce the number of mice used. This tissue includes analysis of normal and diseased post mortem brain tissue that can be used for the determination of GPCR expression levels and testing the activity of GPCR drugs in a disease context.

3. We are using well described protocols such as elevated plus maze, novel object recognition and pavlovian fear conditioning that we are highly experienced in and therefore requires little to no training and few pilot studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have excellent management systems and data-bases in place to ensure efficient breeding and husbandry of the mice.
Where possible we also share tissue amongst users and importantly co-ordinate studies to most efficiently use mice. We also have a large tissue archive which is well indexed and stored.
These approaches will reduce the prospect of overbreeding.

A retrospective assessment of reduction will be due by 20 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

GPCR mutant mice
These will be used to directly determine the role of GPCRs in normal physiology and in disease. In particular we have developed mutant receptors that cannot be activated by the chemicals produced naturally by the body but instead be activated by drugs. In this way we can not only test the role of the target receptors in normal biology but importantly test the action of drugs that work by activating these receptors. Also we have developed GPCR mutant mice where the receptor is restricted in the number of signalling pathways that can be activated. Such receptor mutants can be used to determine the biochemical and signalling pathways used by receptors to mediate clinically important effects and distinguish these from pathways that lead to adverse responses.
These models are designed to directly test the modes of action of GPCR drugs. Without these models wild type

animals would be used. The issue is that wild type animals will display an array of responses a number of which are adverse such as pain, seizures and adnormal behaviour. These adverse responses are often due to off-target drug activity. The animal models used here reduce off-target activity and therefore reduce adverse responses.
Animals will be monitored and adverse responses measured using the criteria set out in the section keeping animals alive.
Neurodegeneration models
These strains will be used to determine the impact of GPCRs in the progression of disease and disease symptoms and whether targeting these receptors can modify disease.
Animals will be monitored and adverse responses measured using the criteria set out in the section keeping animals alive.
We will also use a range of genetically modified mice to investigate how GPCRs work in the brain and how these receptors relieve symptoms of neurodegeneration and slow disease progression.

Why can’t you use animals that are less sentient?

Where possible we will use early life stages (e.g. mouse embryos and neonates) to generate neuronal cultures and terminally anaesthetised animals for histology (e.g. perfusion fixation). However the neuroscience projects require models that most closely resemble human physiology and be models that can be genetically manipulated in this case mice are the most appropriate. Also we wish to test the action of receptors and drugs that might lead to new drugs for human use. The receptors and receptor system need therefore to closely relate to humans and therefore mammalian systems such as mice are the most appropriate.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We are always looking for further refinement of procedures. For example we have been undertaking a study to determine biomarkers of neurodegeneration that can be used to establish early on in disease if targeting our receptors of interest impacts on disease before clinical signs appear. Hence, we will pilot experiments, reading the literature and discussions with collaborators be looking to minimise welfare costs.
Specially, if biomarkers can be identified then we will significantly reduce the number of prion infected animals that progress terminal stage of disease. Currently this step 1 is classified as severe since a proportion of these animals will be progressed to terminal end points. The biomarkers would predict terminal end points before the animals reached a severe category. Hence determination of disease biomarkers would reduce the number of animals progressing to a severe category.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Our primary published source of guidance on 3Rs is via the national centre for replacement, refinement and reduction in animal research (NC3Rs). This organisation publishes regularly on guidance for researchers. The European Medicines Agency also publish excellent practical guidance on 3Rs. We also pay particular attention to the peer reviewed scientific literature for further methods to refine our protocols.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We have regular up-date sessions and training in new approaches run locally and nationally. We also keep abreast of the published literature and share good practice locally. Importantly, we also have expert collaborators that share good practice and we are always looking at new methods to improve our 3Rs.
A retrospective assessment of refinement will be due by 20 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

30. Detection of genotoxic substances

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  0
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs,foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

Genotoxicity studies are necessary for hazard assessment and are legally obligatory. The overall aim is to ensure that both new and existing chemicals do not present a potential carcinogenic, inheritable or otherwise toxic hazard to the public, patients and the environment.
This project will aim to evaluate the effects of chemicals, human and veterinary drugs, medical devices, food additives, biocides and plant protection products to see if they damage cells in animals. If substances damage cells it can lead to the development of diseases like cancer. The tests carried out in this project will identify genotoxic levels of these substances, to enable decisions about how hazardous they are, and so people can be better protected. These tests are legally required before products are exposed to the public by Governments around the World. **A retrospective assessment of these aims will be due by 03 January 2026**
The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A key benefit of this programme of work is the provision of safety data to facilitate sound regulatory decisions when assessing the risks to which humans when the test substances are produced, transported or used.
This work is vital to the development of safe substances (chemicals, human and veterinary drugs, medical devices, food additives, biocides and plant protection products) that people will come into contact with.

What outputs do you think you will see at the end of this project?

The output of this project will be the provision of safety data to facilitate sound regulatory decisions when assessing the risks to which humans, animals, plants or the environment are exposed when substances are produced, transported or used.

Who or what will benefit from these outputs, and how?

The overall benefit is to ensure that both new and existing chemicals do not present a potential carcinogenic, inheritable or otherwise toxic hazard to the public, patients and the environment.

How will you look to maximise the outputs of this work?

Development and validation of new tests or modifications to existing assays will lead to an improved battery of tests for hazard and risk assessment. In addition, many new tests or modifications may allow more thorough assessment of genetic hazard in one step, thus eliminating the need for extensive further testing and reducing overall animal usage. Wherever possible, data from multiple end points will be obtained from the same animal.

Species and numbers of animals expected to be used

- Mice: 5700
- Rats: 20000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult rats and mice are the species specified in the regulatory test guidelines.

Typically, what will be done to an animal used in your project?

Test substance will be administered by oral, intravenous or parenteral routes of administration. Surgical procedures will be used if administration is by continuous intravenous infusion. The duration of dosing is typically 1-3 days, but may be up to 1 month to meet the scientific aim of the study; some studies may require a longer dosing period in order for adequate characterisation of dose response. Dosing may include wash out periods or interrupted dosing intervals.
Animals may be housed in specific cages for the collection of urine and faeces.
Blood samples may be taken by insertion of a hypodermic needle or from cannulae surgically implanted into blood vessels.

What are the expected impacts and/or adverse effects for the animals during your project?

In the majority of animals, there will be minimal adverse effects. Some animals are expected to exhibit some toxicity, but in these instances effects such as signs of pain or discomfort, or abnormal behaviour will mostly be short-lived, lasting for a few hours after dosing.
In range finding studies, some animals may experience severe toxicity, which could include mortality, in those instances where the effects of the substance are not yet known. Any animals showing severe signs will not be maintained and will be humanely killed at the earliest possible point. Most range finding studies will result in no more than moderate adverse effects that are tolerated for the duration of the study.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Based on previous experience, it is expected that approximately 80% of animals will be in the mild severity category, approximately 15% will be in the moderate severity category and approximately 5% will be in the severe severity category.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 03 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Regulatory guidelines require the use of animals to investigate genotoxicity. The *in vivo* tests are conducted because some agents are mutagenic *in vivo* but not *in vitro*. The *in vivo* tests also include additional relevant parameters such as absorption, distribution, metabolism and excretion, which may modulate the genotoxic effects of a test substance.

Which non-animal alternatives did you consider for use in this project?

The ECVAM database and other literature searches were conducted to determine if any non-animal alternatives were available. However, the animal study is preceded by an *in vitro* assay, the results of which are used to optimise the design of the animal study.
All studies will be assessed to verify that there is a need to conduct the study and that there is no other data or approach that could avoid *in vivo* tests.

Why were they not suitable?

No non-animal alternatives are accepted alone by Regulatory authorities. However, if there is data or previous test results available that mean *in vivo* tests are not required, procedures will not be conducted for that purpose.
A retrospective assessment of replacement will be due by 03 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These are based on the numbers used during the last five years and the expected demand for this service.
The requirements of the various tests and regulators will be followed so that only the required number of animals are used in each study.

What steps did you take during the experimental design phase to reduce the number of animals being

used in this project?

Standard study protocols are ethically reviewed against the known guidelines/recommendations, AWERB policies and any recent 3R's refinements. The reviews are undertaken by a committee comprising the Project Licence holder, NVS, NACWO and a lay person as well as other interested parties. Any study requested by a Sponsor that deviates from the approved standard design or involves a new animal related procedure or methodology is subjected to a specific ethical review by the AWERB to ensure all ethical considerations have been taken into account. Statistical advice from in-house experts may also be requested.
ICH promotes the assessment of genotoxic effects by including the relevant end-points into other toxicity studies that are required for regulatory submission. This has clear advantages in terms of animal reduction, however, the study designs must meet specific requirements, so that they are acceptable to regulators and that further animals studies can be avoided.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Regulatory guidelines define the minimum testing requirements for adequate data/statistical analyses for the majority of assays and the study plans used generally adhere to these guidelines/recommendations. Where no guideline recommendations exist, animal numbers are selected on the basis of published literature and/or internal validation data that identify the minimum number of animals required for adequate statistical power.
We will always seek to minimise the use of control groups and multiple dose levels, where this is appropriate.
A retrospective assessment of reduction will be due by 03 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rodents, specifically rats and mice, are the species of choice for these assays, as these species have the largest historical database available and have been routinely used in Genetic Toxicology testing for several decades. Genetically altered animals may also be used. Only animals without an adverse phenotype will be used.
The methods used (dosing, blood sampling techniques, handling and restraint, use of analgesia) will be reviewed during the life time of the licence and any refinements developed will be implemented.
Why can't you use animals that are less sentient?

The species used is generally the same as the rodent species used for the general toxicology studies. The toxicology and/or toxicokinetics from the general toxicology studies can then be used to inform dose selection, sample times etc for the genotoxicity tests, thus reducing animal usage. Species selection may also be driven by known absorption, distribution, metabolism or excretion (ADME) differences between rodent species.
The Regulatory test guidelines require the use of young adult animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Under current regulatory requirements, it is necessary to perform genetic toxicology studies at doses that produce a degree of toxicity, usually in terms of presence of clinical signs and body weight change. Industry guidance and best practice about dose setting are followed, to avoid unnecessary toxicity. All procedures are kept to the minimum commensurate with the study objective. Best practice guidelines for all animal care and use are followed.
Dose range finding studies may be needed to establish suitable dose levels for the genetic toxicology study. Once signs indicate that a dose is unsuitable for genotoxicity assessment, further dosing of the sex/group is usually halted and after any necessary observations have been performed, humane endpoints are applied. Additional observations are included as necessary to closely monitor the condition of the animals. Industry guidance and best practice about dose setting will be followed, to avoid unnecessary toxicity and we will use the results of other studies and data wherever possible, to avoid having to run range finding studies.
Wherever possible, we will use doses that do not produce toxicity e.g. where it is known that a limit dose can be used, or that higher doses are not required. Signs of toxicity are typically seen in mid/high dose animals. Usually these are mild/moderate, however, animals may show significant adverse effects and in this case action will be taken to alleviate signs, such as temporary or permanent withdrawal of the animal from dose, reducing the dose if appropriate or via the application of humane endpoints.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will monitor for updates and other refinements and guidance through the life of the project and apply wherever possible.
Experiments will be conducted in compliance with OECD Test Guidelines and ICH S2(R1). Dose setting will be determined by the requirements of these test guidelines.
For any surgical interventions, then the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) will be followed.
For blood sampling and dosing then the following guidelines/literature will be followed:
First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).
A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23 (2001).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through the regular review of non-animal alternative developments / resources, attendance of scientific conferences and animal welfare forums and reviews of scientific literature. **A retrospective assessment of refinement will be due by 03 January 2026**

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

31. Determining the therapeutic potentials of cardiac signalling molecules

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

therapy, cardiac hypertrophy, cardiac arrhythmias, signalling molecules

Animal types	Life stages
Mice	neonate, adult, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To determine the therapeutic potentials of identified signalling molecules in heart disease conditions.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This project will advance our knowledge of cardiac signalling molecules and their therapeutic potentials in cardiac disease conditions, which will translate into new therapies **What outputs do you think you will see at the end of this project?**

1. New information: the scientific community's knowledge about the cardiac signalling regulation in both health and disease.

2. New products: potential new drugs for treating heart disease, in particular the new drugs for treating heart failure, inherited hypertrophic cardiomyopathy (HCM) and ventricular arrhythmias such as catecholaminergic polymorphic ventricular tachycardia (CPVT).

3. Publications: Publish the findings in academic journals. The information is likely to be of interest to clinicians, physiologists with an interest in intracellular signalling and their roles in cardiac diseases.

4. Model system or new technology: through this project, we will be able to identify/establish suitable animal models for studying cardiac diseases and may generate new valuable technologies for the scientific communities.

Who or what will benefit from these outputs, and how?

1. The scientific community: new information, new knowledge, new animal models 2. Patients: new drugs such as novel drugs for the treatment HCM, heart failure, CPVT.

3. NHS and doctors: new treatment for HCM, heart failure, CPVT.

How will you look to maximise the outputs of this work?

1) dissemination of the new knowledge generated from the project via national and international media or newsletters, etc. 2) national and international collaboration with academic researchers; 3) publication of successful and unsuccessful approaches.
Species and numbers of animals expected to be used

- Mice: 9000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Models of heart disease including cardiac hypertrophy and heart failure in small rodents, particularly mouse models have been very useful for both mechanistic research and the assessment of pharmacological therapies for several decades. In addition, genetically modified mouse models have been widely used in heart disease research. Many of these genes being modified have proved to be crucial in the initiation and progression of heart disease.
Choice of life stage: For breeding protocol 1, it requires neonate, adult, juvenile, pregnant adult life stages of the mice for generating, characterising and maintaining the colonies, while for Protocols 2 and 3, adult, juvenile stages are required for studying the disease process and treatment that are relevant to the disease process in human life stages.
The mice will develop cardiac hypertrophy (either those drug-induced or developed spontaneously due genetic defect such as hypertrophic cardiomyopathy) that can be detestable by echocardiography or magnetic resonance imaging (MRI) but without presence of any clinical symptoms. Any animal showing clinical symptoms will be killed humanely.

Typically, what will be done to an animal used in your project?

1. multiple administration of control substances or substances that cause gene deletion/inducting/hypertrophy or treatment agents by injection, oral route (e.g. gavage) or osmotic mini pump delivery

2. one or more ECG or Echocardiography.

3, one or more recovery general anaesthesia

What are the expected impacts and/or adverse effects for the animals during your project?

1. Develop cardiac hypertrophy without clinical symptoms

90%
2. Wound break down may occur

<5%
3. Damage to the trachea/oesophagus from incorrect use of the Dosing cannulae or Misplacement of the feeding needle resulting in fluid being delivered to the lungs.

<1%
4. dehydration, local irritation, infection.

<5%

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

1. 10% (<200 animals out of 2000 animals in Protocol 2), Moderate 2.

10% (<200 animals out of 2000 animals in protocol 3), Moderate

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

To assess the development of disease or efficacy of treatment, animal models are essential to achieve this goal.

Which non-animal alternatives did you consider for use in this project?

i) we will use patient specific iPSC-CMs for modelling cardiac hypertrophy and for mechanistic studies and for testing and developing new compounds developed based on our identified signalling genes in this project and for future drug development.

ii) Mathematical simulations to construct cellular, tissue and organ models to link cellular and molecular mechanistic alterations to pathophysiological and structural phenotypes at organ levels.

Computational modelling of the heart is now recognised as a powerful technique in the detailed investigation of cardiac behaviour. A biophysically detailed computer model of the heart, the virtual heart, provides a powerful tool for simulating drug-ion channel interactions and cardiac functions at normal and disease conditions, and therefore forms a powerful platform for drug cardiotoxicity screening.

Why were they not suitable?

i) we will use patient specific iPSC-CMs for modelling cardiac hypertrophy and for mechanistic studies and for testing and developing new compounds developed based on our identified signalling genes in this project and for future drug development.

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Computational modelling of the heart is now recognised as a powerful technique in the detailed investigation of cardiac behaviour. A biophysically detailed computer model of the heart, the virtual heart, provides a powerful tool for simulating drug-ion channel interactions and cardiac functions at normal and disease conditions, and therefore forms a powerful platform for drug cardiotoxicity screening.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The animal requirements will be regularly reassessed to ensure minimal animal usage and to maximise our statistical analyses throughout the course of our projects. ANOVA test, Factorial ANOVA in particular, will be used in our experiments for measuring the effect of two or more independent variables on the dependent variable between experimental groups (e.g. WT and mutant groups, treated and untreated groups).
Informed decisions to carry out several or all procedures on any given strain of interest will be made based on the data obtained from one or several of these procedures in conjunction with in vitro, cellular, molecular and electrophysiological data etc.
It is expected that we will keep 10 lines of mice, resulting in approximately 5000 mice being bred during this project based on our experience. These mice will be used for subsequent analysis in regulated procedures or to be used in non-regulated procedures including biochemical analysis analysing DNA/RNA/protein extracted from heart tissue, histological analysis and analysis of Ca2+ handling, for example, in isolated cardiomyocytes. It is expected that approximately 1000 mice will be used in nonregulated procedures such as langendorff heart perfusion for cell isolation, cardiac imaging, etc.
It is expected that approximately 2000 mice will be used in physiological cardiovascular analysis. The majority of the studies carried out under this licence will utilise the mouse model for its flexibility in the generation of genetically modified lines. There are some mouse models of cardiovascular disease and cardiovascular conditions.
Up to 10 genetically modified murine lines will be analysed during the duration of project. For each line male and female GM mice and their WT littermates will be analysed; analysis will be carried out at different ages in the mice to determine the progressive effect of the genetic modification on cardiovascular performance. Each line may be expected to undergo serial echo/ECG and pacing analysis.
It is expected that approximately 2000 mice will be used in hypertrophic induction or treatment. Models of cardiac hypertrophy generated by pharmacological or exercise approaches presents no more than moderate severity phenotypes, while anti-hypertrophic treatment mitigates the hypertrophic process should also present no more than moderate severity phenotypes. The experimental design using NC3Rs EDA has been used for calculation of the number of animals needed for each experiment. We have estimated the number of experimental and control groups, the total number of animals used in each experiment and the number of animals in each experimental group, and the number of times each animal will be measured; the number of

independent replications of each experiment indicated; any steps taken to minimise the effects of bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. blinding).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

A thorough literature search was conducted to ensure that experiments that will be undertaken do not duplicate reliable data that is already published or present in data/resource repositories. Principles of experimental design have been applied in order to minimise the number of experiments required to achieve reasonable statistical significance and data reproducibility.
Three steps for optimising experimental design have been taken:

1. The study design (justification for how experimental and control groups were chosen) for each project. By using NC3R's EDA, we have made estimation of calculation of studies

2. Avoidance of bias

When two or more treatment groups are compared, the animals in the groups will be in identical environments and be similar in every way apart from the applied treatments. Bias will be minimised by:

- Randomly allocating animals to the treatment groups
- Ensuring that all subsequent treatments (including housing) are applied in a random order
- Ensuring that researchers analysing experimental outcomes are unaware of the treatment received (blinded) until the final statistical analysis.
- Using appropriate number of animals (sample size)
- Controlling inter-subject variation (e.g. using randomisation)

3. Considerations for estimating sample size

- Variation is controlled by randomly allocating animals of similar genotypes, of a similar weight and age, which have had a similar environment throughout their lives. Variation due to circadian rhythms or fluctuations in the environment can often be reduced by appropriate experimental design, for example by using blocking factors.

- Measurement error will be minimised by careful technique and good instrumentation and by blinding the researcher to treatment allocation.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will take rigorous measurements to optimise experimental design, pilot studies and computation to minimise unnecessary over-use of animals. The majority of the lines are from our current licence. For the new lines required, where possible we will import previously generated GM mouse lines from

colleagues/collaborators/resource centres. Stock levels of mouse strains will be set to minimise animal breeding whilst at the same time ensuring that given strains are not lost. Colony size management will be controlled by collaboration with the animal house staff and by the use of breeding calculations.

Tissues/embryos, DNA/RNA and protein samples obtained from experimental animals/embryos will be preserved, usually by storing in liquid nitrogen or at -80 C, to allow, where scientifically justified, use in other experiments by ourselves or other scientists who may request such samples.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mouse is the system of choice for experimental genetic modification as applied to mammals and the only appropriate animal model for studying hypertrophic mechanisms and treatments. Mice are a wellknown source of information within the laboratory field and the techniques used here are the most refined at the moment. Breeding and maintenance of genetically modified mice is not expected to suffer any more than mild severity. The administration of transgene inducing or deleting agents will be carried out via intraperitonea injections or oral gavage as the most refined methods.
Physiological analysis: Animals are expected to suffer no more than moderate harm due to the nature of the non-invasive technique of ECG and echocardiography which are the most refined method for this procedure. Expected actual severity is mild.
Hypertrophic models: to generate drug-induced hypertrophy mimicking clinical condition, administration of potential hypertrophic agents and/or substances to potentially inhibit hypertrophy by one of the surgical routes is required. For characterisation of the phenotypes, ECG, echocardiography, magnetic resonance imaging (MRI) and other measurements are needed. Animals are expected to suffer no more than moderate harm due to the nature of the non-invasive technique of ECG and echocardiography. MRI is only taken as a terminal procedure. Implantation of osmotic mini-pump is a well-established technique in the field for delivery agents (eg. Ang II) for inducing pharmacological hypertrophy and has been used in my group routinely for many years. The animals are well tolerated. The antihypetrophic treatment will be mainly delivered by oral gavage or food supplementary whenever is possible. These are refined routes for the treatment.
Why can’t you use animals that are less sentient?

We analyse the consequences of specific genetic modifications for cardiac signalling pathways for cardiac hypertrophy that mimics human conditions. These only occurs in mammals so less sentient model organisms are not an option.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will keep up to date with all procedures and their refinements and will attend any

relevant workshops.

Minipump implant will cause no more than moderate suffering but is not expected to cause

harm. Animals are expected to make a rapid and unremarkable recovery from the implantation

procedure. Analgesic agents will be administered.

Humane endpoints
A key component of refinement is the implementation of humane endpoints.
Welfare assessment protocols and score sheets will be useful tools to monitor adverse effects and determine when humane endpoints have been reached.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

NC3Rs and ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By regularly attending the NC3Rs days available.
Regular contact with transgenic groups within our institution allow us to update each
other with advances in techniques and training.
Departmental animal welfare meetings held termly allow us to communicate any 3Rs implemented within our

group and invited members including NACWOs and NVS ensure that any new advances are disseminated.

Home Office

NON-TECHNICAL SUMMARY

32. Developing Nanoparticles for Brain Drug Delivery

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b) **Key words**

nanoparticles, Blood brain barrier, zebrafish, behaviour

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Efficient delivery of compounds to the brain represents a major challenge in the discovery and development of new therapeutics for the treatment of diseases affecting the Central Nervous System (CNS). Because of the presence of the Blood-Brain Barrier (BBB), only 3–5% of brain-directed pharmaceuticals are able to reach this

area in vivo and have been introduced into the market. The use of very small materials (i.e. in the nanometer range) as drug carriers represent a very promising approach in this field since they have shown a series of additional advantages. This project aims to identify novel nanocarriers able to cross the BBB and suitable for drug delivery. Specific objectives are:

1. to identify novel nanoparticles as candidates for CNS drug delivery

2. to determine the in vivo distribution of these candidate nanocarriers

3. to evaluate potential long-term consequences of short-term nanoparticle exposure (effects on behaviour).

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This project offers the opportunity to identify novel nanomaterials to be used as drug carriers capable of crossing the blood brain barrier for the treatment of brain disorders. The biosafety and ability to target the brain will be verified before moving to clinical trials.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The project uses only zebrafish as an animal model. It is envisaged to use up to 12000 animals over a period of 5 years.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Exposure to nanoparticles may cause signs of mild harm (e.g. oedema, reduced swimming at larval stages), so animals at all the stages will be assessed frequently (i.e. at least twice per day) for morphological and behavioural phenotypes. Any animal showing signs of harm will be killed by schedule 1 method. In addition, mild stress as a result of single housing and behavioural assessment may occur from the behavioural analysis. Animals will be killed at the end of the procedure or, where non-exposed animals have suffered no more than mild severity procedures during the behavioural assessment, and are not suffering or likely to suffer, they may be maintained for breeding purposes.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

It is not possible to determine the actual suitability of these novel nanomaterials as drug carriers to the central nervous system without in vivo studies. In addition, an assessment of potential behavioural changes can be only achieved through the use of an animal model. Zebrafish are the most suitable animal model to use because they are non-mammalian vertebrates that have been shown to have a translationally relevant behavioural repertoire. Similarly, it is not possible to assess the ability of nanoparticles to target specific organs or tissues in vivo without an animal model. Considering the BBB and the central nervous system are targeted, zebrafish represent a very reliable model because their BBB is both structurally and functionally similar to that of mammals.
However, all in vivo analyses will be preceded by in vitro (3D human BBB model) analysis to confirm effects on cell survival or migration before testing in vivo. All nanoparticles will be tested for harmful effects on cells in culture prior to use in animals and, in case of damage, they will not be administered in vivo

Reduction

Explain how you will assure the use of minimum numbers of animals.

Results from past work from our lab and elsewhere will be used to conduct statistical power tests to calculate the fewest number of animals required in order to achieve our objectives as stated in project plan.
Within our facility we have experienced fish technicians who regularly monitor the health of the fish, and a fully trained NACWO. These individuals are on hand to provide experimental design opinions as well as remove animals from the project if they are suffering any pain or distress, or show any signs of harmful abnormalities.

Nanoparticle preparations promising as drug carriers will initially be investigated for harmful effects in vitro. Any chemical showing to damage cells in culture will not be taken forward. Effects of chemicals on development of wild-type zebrafish embryos will be assessed after 24 hours of embryonic exposure (at 48 hours post-fertilisation). Again, any chemical that induces abnormal development will not be taken further and assessed for neither effects on behaviour nor localisation within the body. Larvae will be assessed twice daily for signs of adverse effects of exposure to these vehicles (e.g. swimming behaviour, gross morphology, oedema). Any nanovehicle inducing signs of harmful effects will not be taken further.
When the analysis will require the use of fluorescence microscopy, a single animal can be assessed at several different stages post-injection (fluorescence imaging is non-harmful).

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Zebrafish are the most appropriate model species as they are vertebrate with a high degree of conservation of human brain organisation and functions making results found in zebrafish of translational relevance.
Furthermore, they are the vertebrates with the lowest neurophysiological sensitivity likely to yield results relevant to the human condition.
Use of initial pilot studies in 24hr larvae will enable any unexpected harmful effects to be detected at the earliest stages. Only nanoparticles shown not to induce any damage at early life stages will be taken forward to older stages.

We primarily use locomotion behaviour, shoaling and appetitive learning paradigms as our means of assessing behavioural phenotypes. Appetitive paradigms can be considered less severe than those using aversive learning. All animals shall be killed at the end of a protocol unless they were only subject to control behavioural analysis that consists merely of placement in a tank with specific markings and the performance of locomotor or appetitive learning tasks (approaching lights for food). These control fish may then be returned to the breeding programme but will not be used for further behavioural analysis.
Measures of anxiety use exposure to forced light transitions to induce a startle response in 5dpf-10dpf larvae. In adults anxiety is assessed using a tank diving assay, a fish version of the open field test, where more anxious animals tend to spend more time near the bottom when transferred to a novel tank. These stressors are considered the mildest capable of inducing a robust response.
The project researcher holds a PIL and will monitor the fish for signs of harm regularly- twice daily. Within our facility we have 2 experienced fish technicians who hold PILS working on this and related projects and who regularly monitor the health of the fish in addition to a fully trained NACWO. They are on hand to remove animals from the project if they show any harmful abnormalities or signs of distress.
Regarding determination of the distribution of nanoparticles, the big advantage of using larval zebrafish lies in the optical transparency of the embryos and larvae which allows the tracking of nanoparticles by using non/invasive imaging systems.

Home Office

NON-TECHNICAL SUMMARY

33. Developing safe and efficacious cell-based therapies for kidney disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b) **Key words**

kidney injury, regenerative cell therapy, preclinical imaging, safety and efficacy, immunomodulation

Animal types	Life stages
Mice	adult, pregnant, aged, embryo, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to develop therapies to treat kidney disease, using mouse models of acute kidney injury and chronic kidney disease. For this purpose, we aim to assess efficacy and safety of cells or their products as regenerative medicine therapies

A retrospective assessment of these aims will be due by 02 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Acute kidney injury (AKI) and Chronic kidney disease (CKD) affect large numbers of patients, particularly adults but also children, and are frequently associated with underlying health problems including cardiovascular disease and diabetes.
AKI is associated with an increased mortality rate and 20% risk of progression to CKD. Treatment of AKI is limited to management of blood pressure, fluid and electrolytes, but renal replacement therapy can be needed. CKD can progress to end stage renal disease which means that dialysis or transplantation are the only remaining treatment options.
Regenerative medicine therapies, including cells or their products, have emerged as a promising avenue for the treatment of AKI or CKD, but our understanding of the mechanism of action and the safety of these cells or their products remain limited. This programme of work will address these points in preclinical studies in mice of various ages (including aged animals), and thus contribute further important knowledge towards the development of safe and efficacious regenerative medicine therapies for treatment of patients.

What outputs do you think you will see at the end of this project?

This project will provide a number of outputs, including:

- A more thorough and detailed understanding of the interactions between RMTs, damaged kidney and the immune system, including their mechanism of action, to promote or enable therapeutic efficacy in mice with kidney injury, and the influence of ageing on these processes. This may include novel insight into molecular and cellular steps that could be clinically exploited for therapeutics.

- The development of novel preclinical imaging assessment methods of renal function and tissue integrity to determine efficacy of RMTs in a non-invasive, longitudinal fashion that can be clinically translated. This includes the development of novel dyes/compounds that can be utilised in these assessments.

- The development of novel imaging tools and cell labels for preclinical imaging to track cells in order to determine their safety, both in the kidneys but also off-target in other tissues, since cell administration may be systemic and/or can lead to off-target growths. This involves the development of novel models to interpret and analyse preclinical imaging data.

- Multiple research publications in peer reviewed journals. We have a strong publication record in our team, with 2-4 publications per year in recent years. We strive to generate publications from our funded studies, not only for the benefit of the young researchers involved, but also to improve general understanding and knowledge in the field.

- Scientists with unique training and expertise in various techniques including surgery, colony maintenance of wildtype and genetically altered animals, use of a range of preclinical imaging modalities, data analysis. These skills will be invaluable for the research careers of the staff and students involved in this programme of work.

The long-term aim of this programme is to generate novel therapeutics that can be translated to the clinic for the benefit of patients with kidney injury or kidney disease.

Who or what will benefit from these outputs, and how?

Short term beneficiaries:
- The renal research community as well as the regenerative medicine research field, including the area of in vivo imaging and image analysis. Our research findings will be communicated to these communities via workshop and conference presentations, which will lead to exchange of ideas and cross-fertilisation of outcomes, ideas and concepts. The research communities will also benefit from research publications generated from our findings, with the potential to stimulate further research.

- The research community will directly benefit, in the short to medium term, from access to novel preclinical imaging techniques and analyses that we will develop during this project, including data on novel dyes and their analysis and interpretation.

Medium to long term beneficiaries:
- This will include the research team as short-term outcomes will build further expertise of the entire team, leading to continuity and consolidation as well as refinement of techniques.

- The research field (both renal, regenerative medicine as well in vivo imaging) will be beneficiaries because accumulation and consistency in outcomes will consolidate the reliability and reproducibility of our work, leading to an improvement in standards.

- Clinicians (renal and others) will benefit from access to improved understanding of mechanisms of action of RMTs and their safety. We expect that our findings will be important contributors to the development of clinically relevant RM therapeutics for treatments in renal patients that can be tested in clinical trials, as well as other diseases where RMTs have shown promise.

- The public will benefit from interactions amongst the above beneficiaries as well as their disseminations and progress in the development and anticipated validation of RMTs. This includes patients with kidney disease (AKI or CKD, possibly renal transplant patients) who would benefit from the development of RMTs as therapeutics that can be tested in clinical trials.

How will you look to maximise the outputs of this work?

Our outputs are maximised by interactions with colleagues from preclinical and clinical research fields, as well as interactions with patient representatives. We have ongoing international and national collaborations. We are strongly engaged with the UK renal research community and a large number of charities. Through these interactions, we reach a wide audience of specialist preclinical and clinical researchers and practitioners as well as lay people.

We maximise our outputs through attendance at workshops and conferences, and most importantly through publication of our findings in open access journals, including the use of pre-print serves (e.g. bioRxiv). We are keen to publish unsuccessful approaches, and datasets are made available after publication.
We have a range of international and national collaborations which arise from our active engagement with the national and international research field of renal regenerative medicine and preclinical imaging. This involves SMEs that are involved in the improvement of imaging modalities, dyes and analyses.

Species and numbers of animals expected to be used

- Mice: 4450

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We want to develop treatments for kidney disease or injury that could in the future be used in patients. We test cells or cell-derived substances as treatment, and use animals in order to find out which are the most successful cells or substances to reduce the injury in the kidneys. We use mice because their kidneys work very similarly to those in humans, and show similar injury response which we use to determine levels of damage, but also because mice are the simplest animal model with this level of similarity to humans. Therefore, mice are the most appropriate animal to achieve our aims. Because kidney injury can occur in younger and middle aged people, but also in older people, we use adult mice, but also aged mice.

Typically, what will be done to an animal used in your project?

Animals in this project may be part of studies that optimise conditions, or be part of a larger study to test and determine the success in treatment of the cells or cell-derived substances. With these studies, we also wish to understand how the successful treatment works, and where the cells that we use as treatment, go in the body. During either studies, most of the mice will get a kidney injury which has similar clinical signs to kidney injury in patients. This can be a severe acute kidney injury caused by injection of a drug, or by surgical induction under general anaesthesia. During the surgery, the blood flow to one or both kidneys is halted for a pre-determined time period in order to establish the desired level of injury in the organs. The mice will be unwell for a period of 2-7 days, but recover. Some mice will be treated so that they develop chronic kidney disease, which is milder in its onset but can show clinical symptoms for longer; mice may be monitored for this condition for up to 6 months. Some of the mice in either injury condition will receive cells or cell-derived substances as test treatment and we will monitor and measure clinical signs of progression of the injury response with our without treatment using the most non-invasive approaches possible that have also clinical relevance. These approaches involve injection of small volumes of contrast substances that can be detected in imaging instruments to determine how well the kidneys function. These steps are typically performed under general anaesthesia. These measurements can take place repeatedly over the study period but at the most appropriate times to reduce harm to the mice.
In some of the mice, we will have removed specific cell types of the immune system. We know that the immune system plays a role in the development of kidney injury and the limited natural healing process that can take place, and by removing certain immune cell types we can determine whether they are essential for development of the disease or the treatment process.

What are the expected impacts and/or adverse effects for the animals during your project?

The experimental conditions may lead to loss of weight in mice that have kidney injury. The treatments which lead to kidney injury, will lead to the most critical loss of weight, but other steps, including imaging assessments under anaesthesia, can also lead to weight loss. Because of the need for clinical signs of kidney injury in order to assess the effect of the treatments, these adverse effects are necessary.
Aged mice may be more frail than healthy adult mice and their treatment regime during the steps of protocols will be adjusted so that they are milder without preventing us from obtaining relevant information on the same question of kidney injury and effectiveness of treatments with cells or cellderived substances.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Two of the protocols have a severity level of moderate, but most mice in these protocols will experience mild conditions. In the one moderate protocol we may breed genetically modified mice which may show signs of moderate health problems, however, ageing mice will also be generated under this protocol, which can lead to moderate severity in their clinical signs. Overall, we expect that not more than 10% of the mice under this protocol show signs of moderate severity.
In the other moderate protocol we optimise conditions for removing specific immune cells. This can involve the development of clinical signs of moderate levels of severity in the mice, caused by toxicity of some of the agents. After optimisation of the treatment regime with agents used to remove immune cells, no adverse effects have been reported. Therefore, we expect that no more than 10% of mice under this protocol show signs of moderate severity.
Three of the protocols have a severity level of severe. This includes two optimisation protocols where we will optimise conditions to induce kidney injury. We expect that up to 50% of mice in these protocols will experience signs of severe severity. However, we will use small groups of mice for these optimisation protocols so overall numbers of mice experiencing severe severity from these protocols is small.
The experimental protocol with severe levels of severity can also lead to up to 30% of mice experiencing this severity. In this protocol, mice may experience kidney injury which can lead to loss of weight, but treatment with cells or cell-derived substances or modification of the immune system may alleviate the injury level. Some of these mice may experience moderate levels of severity. Also in this protocol included are mice that are not experiencing kidney injury based on experimental design of cell tracking in non-injured animals.
Our careful optimisation approach, combined with monitoring of the wellbeing of the mice throughout, should allow us to minimise adverse events.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 02 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The conditions that contribute to the development of kidney injury and possible treatment cannot currently be

reproduced in the non-animal setting since it involves the effect of blood flow, immune cells and other physiological factors beyond the interactions of cells inside the kidneys. Mammalian physiology and anatomy are complex and other organs may also contribute to the injury and any beneficial treatment response. Any beneficial treatment response observed in a non-animals condition may have to be validated using animals models before treatments can be trialled in patients.

Which non-animal alternatives did you consider for use in this project?

In vitro (cell culture) and ex vivo (kidney slice) systems offer some opportunities to replace the use of mice. Cell culture work is being performed to study effects of therapeutic cells on cells of the immune system, but only deliver limited information due to the isolated setting. Kidney slice models require optimisation of set up which we are currently trying to obtain funding for. However, both approaches will be complementary rather than full replacements of animal studies.

Why were they not suitable?

In vitro approaches cannot reproduce the entire disease setting and physiological condition of the animal. These are important aspects that contribute to the injury and treatment response. This includes interactions with other organ systems and cells as well as physiological parameters like blood flow to the kidneys. Although cell-based studies produce important information in their limited format on the response of isolated cells to specific conditions, a whole range of these conditions are not being assessed. In kidney slice models, a more complex system of various cell types and interactions can be replicated, however, they still lack the intricate interaction between various organs and systems in the animal.
Since the aim of our work is to determine the efficacy and mechanism of action of cells or cell-derived substances within the animal to kidney injury, we need to consider that these processes in the patient take place in a complex environment and that the in vitro or ex vivo experiments cannot completely replicate the animal model.
A retrospective assessment of replacement will be due by 02 May 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

My experience in this area of research over the last 10 years allows me to estimate with confidence the mouse numbers needed for this programme of work. Animal numbers are determined based on currently funded studies and the experience with previous project licences. These are based on effect sizes (including variances) from previous work, and also considering adverse effects.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The studies funded for this programme of work will have undergone peer review which includes providing detailed statistical analysis and power calculations. We have made use of the NC3Rs experimental design assistant but also other online power calculation tools (G star power etc) to design our studies and power our experiments so that we can achieve sound primary outcome measures. We base calculations on group sizes on estimates of effect sizes from our previous work, including preliminary data, and on published studies.
We frequently perform longitudinal studies which for example include multiple measurements of renal function parameters at various time points in the same animal. These longitudinal assessments allow for reduction of animal numbers since it reduces end point analysis at these different time points.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have optimised experimental design specifically for acute and chronic kidney injury studies where ischaemia reperfusion injury was induced by surgical approach. We recognised that animals undergo larger variability in response unless certain surgical parameters were more tightly controlled (see Refinement). The reduction in variability leads logically to a reduction in animal number as the experimental design calculations will include adjusted effect sizes, since variability in response doesn't need to be addressed in larger group sizes.
We perform pilot studies wherever necessary, especially for crucial experimental conditions like induction of injury and removal of immune cells. Optimal conditions arising from these pilot studies will lead to a reduction in animal number since they reduce variance in the experimental and control groups. This can be further refined after ANOVA-type statistical analysis for follow-on studies.
Post-mortem, tissues and other materials will be used to assess the outcome of the experiments. This may involve re-assessment of data using modelling of outputs using optimised computer programmes, for example by making use of machine learning. We are currently starting a project where this is trialled with histological data obtained from our experiments.
Analysis and interpretation of other digital data is also being constantly optimised to both drive optimisation of interpretation and also develop novel approaches to data analysis which may have relevance for applications in the clinic.
A retrospective assessment of reduction will be due by 02 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In this programme of work, we use mouse models of kidney injury that are very closely replicating clinical symptoms. These injuries are required to achieve our overarching aim of developing therapies for treatment of kidney injury, using cell or cell-based treatments. These injury models are widely accepted as standard for

mouse experiments that replicate symptoms of kidney injury in patients.
The methods used to induce kidney injuries either involve the injection of a nephrotoxic substance or surgery to induce an injury to the kidneys. Mice in these studies may be administered analgesics to reduce any pain, and surgery will be performed under anaesthesia.
We need to induce acute kidney injury in a subset of the experimental animals as we can follow the generally quick injury response over a short period time; in mice where we want to model chronic kidney disease, a less distressing injury is induced and the animal's response followed over a longer time period.
Some of the mice in either experimental treatment group will receive versions of cells or cell-derived substances with the aim to reach an reduction of the level of injury, which should correlate with the level of suffering and distress.
Pilot studies will be performed to optimise conditions of treatment to induce kidney injury, and also removal of immune cells. In order to understand the mechanisms of action that underpin any therapeutic

response of the cells or cell-derived substances, we will study the response of the immune system which is intimately involved in injury and healing responses.

Why can’t you use animals that are less sentient?

In this programme of work, we study effects of cells or cell-based substances as therapeutic treatment in mouse models of kidney injury, mimicking variances of the disease found in patients. The mouse is the simplest model organism that can replicate this disease scenario because it has a very similar anatomy and physiology to the human.
Adult or even aged mice are needed to perform these studies because patients typically fall within this bracket. It is necessary to have fully functional organ systems available in the animal for the studies to yield relevant information, and early embryonic stages would not be providing the required data. It is not possible to perform the studies in terminal anaesthetised animals as various outcome measures wouldn't be able to be assessed, making this an impractical approach.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I have worked with mouse models of kidney injury for 10 years now, and during this time we have developed several models (Adriamycin glomerulosclerosis model, ischaemia reperfusion injury model). We have also established multi-modal imaging in healthy and injured mice during this time.
I have >20 years of experience in mouse models of various diseases, and have used genetic lineage tracing and ablation models under a different project licence. During this time, I have established expertise in assessing and scoring animal wellbeing, which could present stages of disease progression and injury. In order to implement the kidney injury models and other procedures within this programme of work, mice are closely monitored during the critical phases after injury induction in order to maintain the severity levels and determine when humane end points are reached. One of the main predictors is animal weight, and so animals are weighed regularly and their overall appearance monitored every few hours on the day of surgery or other induction of injury, and daily afterwards. This may involve close monitoring of wellbeing during critical phases of injury response by night visits in order to avoid unnecessary suffering of the mice.
We constantly strive to refine procedures to minimise suffering and improve welfare of the animals. This includes regular reflection of steps that could be improved. For example, we have optimised the experimental design for surgical induction of acute and chronic kidney injury, since we recognised that animal groups undergo larger variability in response unless certain surgical parameters were more tightly controlled. These observations and refinements have been published on the bioRxiv preprint server and are currently in revision at one of the leading renal physiology journals. This refinement subsequently leads to a reduction in animal number as the experimental design calculations will include adjusted effect sizes, since variability in response doesn't need to be addressed in larger group sizes.
Previously, we established the use of the ultrasound imaging instrument to refine the administration of cells into the left ventricle in order to avoid misinjections and unnecessary suffering.
We are currently working on a protocol to establish physiological and functional renal parameters (including GFR) as predictors of overall animal wellbeing. We plan to incorporate expertise by BSU staff and grimace scoring into an overall score system that allows us to monitor closely the wellbeing of the mice in an unbiased way.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow LASA guidelines on dosing and administration of agents, and withdrawal of blood.
For the scoring of ageing mice, we follow the guidance by Wilkinson and colleagues (Wilkinson et al., 2020, Lab Anim. 54(3), 225-238. DOI: 10.1177/0023677219865291), and also the internal score sheet that has been set up in collaboration with colleagues at the University with longstanding expertise in research using ageing mice.

We have sent PILs/researchers on international courses to obtain specific training in the models used in this project, including the surgically induced kidney injury model. We have taken guidance from the publication by Skrypnyk and colleagues (Skrypnyk et al., 2013, J Vis Exp (78):50495. doi: 10.3791/50495) and others.
We follow the ARRIVE guidelines for which an update (2.0) was published this year (Percie du Sert et al., 2020, PLoS Biol 18(7), e3000410. doi: 10.1371/journal.pbio.3000410).
We have sent researchers to other laboratories nationally and internationally to learn and improve on techniques that are relevant for this programme of work, for example with regards best practice in using in vivo imaging instruments. As part of the EU Marie Curie training network, that is contributing funding to our work, we are in constant exchange with other researchers in the Netherlands, Germany, Italy and Ireland to discuss and improve our experimental designs and approaches.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our Biomedical Services team is very active in announcing and organising NC3Rs meetings, workshops and conferences. I have attended several local NC3R workshops, and have presented at an international NC3R conference on severe suffering. I encourage members of the team to use the NC3Rs website, communicate information that becomes available, and attend conferences. The animal unit at our establishment is proactive in displaying important publications, posters and other information leaflets that are important for work in the NC3Rs area. The NC3Rs regional manager for the establishment is actively engaging with the animal researchers, and happy to provide support where required.
In our work, we constantly aim to apply the 3Rs principles; this includes continuous reflection on studies and subsequent optimisation so that refinements can be introduced to limit suffering, and also to reduce animal numbers. We are also keen to explore alternative model systems (ex vivo etc) in the laboratory.
A retrospective assessment of refinement will be due by 02 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

34. Development and function of neural circuits underlying perception and behaviour in the mammalian brain

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Mice	adult, embryo, pregnant, aged, juvenile, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Animal behaviour emerges as the product of coordinated activity of neurons with different patterns of gene

expression, synaptic connections, and activity. The aim of this project is to link these levels of analysis and provide a mechanistic explanation for how the brain generates behaviour.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

All the richness of animal behaviour is the product of input-output transformations carried out by individual neurons connected into intricate networks. These neurons come in hundreds of distinct cell types, generated during brain development through the activity of different combinations of genes. The rules according to which different cell types select their input and output connections forms the backbone of the neuronal networks of the brain. Yet we still do not know how these connections are established, and how the resulting networks of neurons give rise to patterns of activity underlying cognition and behaviour.

This project will advance our understanding of how different specialised classes of neurons act together to give rise to perception, memory, decision-making and other elements of high level brain function, and how the specialised properties of these neuronal populations are influenced by cell-type specific gene expression programmes. Although understanding how genes guide the specification of input and output connections of neurons is important in its own right, it will also provide key insights into how disruption of genes leads to circuit deficits underlying the symptoms of neuropsychiatric disorders linked to perturbation of neuronal connectivity, including autism and schizophrenia.

What outputs do you think you will see at the end of this project?

This project will advance our understanding of how networks of neurons within the brain process information to give rise to behaviour and how their connections are established during brain development. This knowledge will be disseminated through presentations at scientific conferences and peer-reviewed publications. In addition, any new techniques, reagents, or software tools generated as a part of this project will be made freely available to other researchers in the field.

Who or what will benefit from these outputs, and how?

Experiments in this project will help reveal how the organisation and function of animal brains enables perception, memory, decision-making and other aspects of cognition. This knowledge will be valuable for several reasons. The neocortex of different mammalian species follows similar principles in its organisation. Therefore, studying how the mouse cortex contributes to these high-level brain functions will advance our understanding of how they are carried out in the human brain.

This project will also investigate how the circuits that carry the computations responsible for cortical functions are established during development. Currently, there is a major gap in our understanding of how molecular mechanisms influence, which synaptic connections neurons make in cortical circuits, ultimately defining the computations carried out by them. It is proposed that many neurodevelopmental disorders, most prominently autism spectrum disorders, are “connectopathies” – consequences of disrupted neuronal connectivity. Experiments in this project will disrupt individual genes during cortical development to identify their role the establishment of neuronal connections. This knowledge will reveal the molecular programmes that orchestrate the development of healthy cortical connectivity and provide insight into how their perturbations give rise to neurodevelopmental disorders.

In addition, in pursuit of the goals above we will develop a new platform that will allow us to systematically examine the consequences of knock-out of many individual genes in parallel. This platform could be applied to other research questions and in other brain regions and so could serve to accelerate research seeking to identify potential targets for treatment of neuropsychiatric or neurodegenerative diseases.

How will you look to maximise the outputs of this work?

Whenever possible, we will seek to maximise the utility of data and tools generated as a part of this project through collaboration with other experimental or theoretical groups, and online distribution of raw data and software tools. We will also make use of preprint platforms, such as bioRxiv, to rapidly disseminate our findings to a broad audience.

Species and numbers of animals expected to be used

- Mice: 17100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The overall organization of the brain is similar across mammals. Therefore, insights gained through studying the structure and function of the mouse brain will help understand how the neural circuits within the human brain give rise to perception, memory, decision-making and other aspects of cognition. In addition, the availability of genetically altered mice makes it possible to measure and manipulate neural circuits with unprecedented precision. Most experiments will be conducted in adult mice. In experiments aiming to understand how the nervous system develops and require access to the fetal or neonatal nervous system, pregnant mice and their embryos or neonates will be used. In addition, mouse embryos or oocytes will be used when required for the development and maintenance of genetically altered mouse lines.

Typically, what will be done to an animal used in your project?

To provide access to the brain in experimental procedures in this project, animals will undergo surgeries under deep anaesthesia, during which devices for recording activity of nerve cells or delivery of substances into the brain may be implanted. All animals will receive pain relief and will be closely monitored during recovery. In most cases, animals will undergo one or two surgical procedures, with sufficient time between surgeries to allow for complete recovery.

In some experiments the animals may be head-fixed to allow recording of neural activity and/or reliable presentation of sensory stimuli. In these cases, mice will be habituated to head fixation and the experimental setup to minimise stress and discomfort.

In some experiments, to motivate animals to perform complex behavioural tasks, animals' access to food or water may be restricted, and food or water rewards will be provided during the experiments. In these cases, the animals’ weight and health status will be carefully monitored, and ad libitum food or water will be provided if any adverse effects are observed.

At the end of experiments or if mice show signs of unexpected ill health, distress or suffering which cannot be ameliorated with minimal veterinary or husbandry intervention they will be humanely killed.

What are the expected impacts and/or adverse effects for the animals during your project?

Pain resulting from surgical procedures may reach moderate severity for short periods of time immediately following surgery. Animals will be closely monitored for signs of pain after surgery and appropriate analgesia will be provided.

Head fixation is expected to result in only mild stress during initial habituation to the experimental apparatus.

Animals under food or water restriction will typically maintain around 85%, but at least 80% of their normal body weight.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

57% of mice are expected to experience mild severity, while 43% are expected to experience moderate severity.
What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are still far from understanding how the circuits of neurons within the brain give rise to perception, memory, decision-making and other aspects of cognition, or how the disruption of these circuits leads to disease. Achieving this understanding requires measuring and manipulating neural circuits in ways that are not possible in humans. Instead, we will focus on mice, which are genetically tractable allowing highly precise measurements and manipulations of neural circuits and the development of disease models, and whose brains are organized according to similar principles to humans.
Which non-animal alternatives did you consider for use in this project?

Computer simulations

In vitro brain organoids
Why were they not suitable?

Computer simulations of neural networks require precise knowledge of the structure of connections between nerve cells and their properties. We still lack sufficient data to reproduce using simulations the behaviour of even the simplest nervous systems, such as those of nematode worms, least of all of the much more complex mammalian brains.
In vitro brain organoids have the potential to recapitulate much of the cellular diversity of the nervous system. However, this project is focused on studying how neurons within the brain process information from the environment and contribute to behaviour, which is only possible in vivo.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals are estimated based on our prior experience breeding genetically altered mouse lines

and with experimental approaches used in the project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Using our extensive experience with experimental techniques and statistical expertise, we will ensure that we use the minimal number of animals to achieve our scientific objectives. We will use state-of-the art methods to measure neural activity of many brain areas or neurons in parallel and develop sophisticated data analyses approaches to extract the maximum amount of value from these results. Whenever possible, we will use within experiment comparisons to maximise statistical power and minimise the impact of external variables. Finally, as a part of this project we will seek to develop new methods that will allow us to measure or manipulate gene expression or neural activity in many populations of neurons in parallel in the same animal, greatly reducing the total number of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies will be used to test the efficiency and applicability of new tools and techniques before employing them broadly to pursue the scientific objectives of the project. Computer modelling will be broadly applied to refine experimental hypotheses. Whenever possible, post-mortem brain tissue will be re-used and shared. Raw experimental data will be stored and re-used for new analyses, whenever possible.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project will exclusively use mice. Surgical procedures will be conducted under deep anaesthesia following aseptic technique and mice will be carefully monitored following surgery to ensure complete and uneventful recovery.

Why can’t you use animals that are less sentient?

While mammals, including mice, share the basic principles of brain organization with humans, the brains of other vertebrates and invertebrates are distinct. For example, the neocortex, which plays a key role in high level cognitive function, is present only in mammals. Carrying out experiments in this project solely in terminally anaesthetized animals is not feasible since studying perception and behaviour necessitates the use of awake animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Appropriate analgesia will be used during surgical procedures and mice will be closely monitored during recovery. In experiments involving head-fixation, mice will be gradually habituated to the experimental setup to minimise stress.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the guidelines set out in LASA Guiding Principles on Preparing for and Undertaking Aseptic Surgery.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will strive to continuously improve our procedures with the help of the biological research facility staff, the Named Information Officer, Named Animal Care and Welfare Officers and the Named Veterinary Surgeons. We also consult the websites of NC3RS and RSPCA to keep up with the latest advance.

Home Office

NON-TECHNICAL SUMMARY

35. Development and function of the immune system

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Immune system, Signal transduction, Lymphocytes

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to understand the biochemical processes within immune cells that control their development, activation, survival, migration, differentiation and function.

Potential benefits likely to derive from the project, for example how science might be advanced or how

humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The immune system is essential for protection from infection by pathogens. An insufficient immune response will cause humans to succumb to infectious disease, whereas an over-active immune response can result in immune pathology during infections which can be a significant cause of morbidity and mortality. Furthermore, over-activity of the immune system to innocuous stimuli results in autoimmunity, again with significant adverse health consequences. Understanding how immune cells function and are regulated is critical basic research that will underpin development of more effective therapies to support or suppress the immune system when it underperforms or overreacts.

What outputs do you think you will see at the end of this project?

The main outputs of this work will be knowledge about how biochemical processes within immune cells control their development, activation, survival, migration, differentiation and function. These will be published in peer-reviewed journals, and the publications will always be open-access and thus available to all to read for free.

Who or what will benefit from these outputs, and how?

The immediate beneficiaries of this work will be other academic researchers studying similar biochemical processes that regulate immune cell function. More broadly, it will benefit immunologists studying how the immune system responds to challenges and how autoimmunity develops. Most importantly, in the long-term, our work will provide the basis for the design of rational therapies that can modulate immune system function, which could be applied for the treatment of autoimmunity, immunodeficiency and immune pathology caused by over-exuberant immune reaction to pathogens.

How will you look to maximise the outputs of this work?

The main outputs from the work will be published in peer-reviewed journals, and the publications will always be open-access and thus available to all to read for free. We will also communicate our work through presentations, by giving seminars at other institutions or through seminars or poster presentations at conferences. Unsuccessful approaches will be discussed openly in appropriate venues, for example at internal meetings. We have an extensive track record of collaboration, helping other groups with more limited experience in these areas of research. We will continue to support such collaborative work.

Species and numbers of animals expected to be used

- Mice: 63000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the animal of choice for these studies because, like humans, they are mammals and thus their immune system is closely related to the human immune system. In addition, the immune system of mice has been studied more extensively that that of any other mammal, and huge numbers of reagents are available. Most of the animals studied will be adults, but some work on the development of the immune system will be carried out using embryos, neonates or juveniles.

Typically, what will be done to an animal used in your project?

The large majority of the animals in this project will be bred in order to generate genetically altered mice and will have no further procedures done to them. These mice will be analysed once they reach adulthood by taking tissues from them for analysis in vitro once they have been killed. For some mice they will be immunised and their immune response will be studied, which may involve taking blood at several time points. In all cases much of the analysis will be done on tissue taken from the mice after they have been killed. Typical experiments may last a month, with the mice immunised at the start, bled once a week for 4 weeks and then killed for final analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Most animals will suffer no adverse effects. A minority of animals will be injected with cells or substances to study the immune system. This will cause transient pain but no lasting harm. A small number of mice will be given viral pathogens which will cause moderate clinical symptoms.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild – 95%

Moderate – 5%

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is not possible to comprehensively study the immune system outside the living organism, since its function depends on complex interactions between many different types of cells located in different tissues of the animal.

Which non-animal alternatives did you consider for use in this project?

It is possible to mimic some aspects of how immune cells behave using established cell lines, however this is very limited. Indeed, such in vitro approaches work best using immune cells taken directly from animals.

Why were they not suitable?

Established cell lines are not able to replicate the behaviour of normal immune cells.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimate is based on our current experience of carrying out similar studies under our current project licence.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The efficiency of animal usage will be maximised by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. This has been greatly facilitated by a custom-built mouse database in which every breeding pair and every mouse born are recorded and through which we can readily monitor the numbers of mice we hold. Many experiments will require homozygous mutant animals. Littermates of these that are heterozygous or wild type will be used as age- and gender-matched controls. This allows optimal use of mouse numbers generated as well as being best scientific practice for the study of genetic alterations.

The experimental design is always based on using the smallest number of animals that are sufficient to answer the question being posed. We expect, from experience, that 6-8 animals per treatment group should be sufficient to obtain statistically robust results. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from the literature).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding strategies are always set up to maximise the number of useful mice from each litter. Wherever possible we will use multiple tissues from every animal, in order to maximise the data obtained from each mouse. Cryopreservation of sperm or embryos will be used to preserve mouse strains, thereby obviating the need for continuous breeding and thus minimizing numbers of mice used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mice since these are the mammal with the best studied immune system. Most of the work will be carried out using tissues from genetically altered moue strains analysed in vitro. Only a minority of the work will require further procedural work, such as immunisation. Where immunisation is needed, the methods to be preferentially used will be ones where there is no lasting harm to the animal, e.g. immunisation with model antigens.

Why can’t you use animals that are less sentient?

Mice are the animal of choice for these studies because, like humans, they are mammals and thus their immune system is closely related to the human immune system. In addition, the immune system of mice has been studied more extensively that that of any other mammal, and huge numbers of reagents are available.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will always choose procedures that cause the least amount of harm. Animals undergoing procedures will be

carefully monitored for signs of any impairment of welfare and, in rare cases, if required will be provided with pain relief.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Publications from the NC3Rs and the Institute for Animal Technology, as well as relevant articles in scientific journals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay up to date via regular communication with animal facility staff at the host establishment, other scientists in our fields, via e-mail and other updates and publications from, and occasional attendance at meetings held by, the NC3Rs, the Institute for Animal Technology, and the International Society for Transgenic Technology, and through regular visits to their websites: https://www.nc3rs.org.uk/3rs-resources https://www.transtechsociety.org/ https://www.iat.org.uk/

Home Office

NON-TECHNICAL SUMMARY

36. Development and validation of animal models for neurodevelopmental disorders

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Rats	pregnant, neonate, adult, juvenile, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The project aims to understand how activation of the mother's immune system by viral or bacterial infection during pregnancy can cause of neurodevelopment disorders in the young, principally schizophrenia. From this understanding, we also aim to develop therapies to treat and prevent neurodevelopmental disorders.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Public health science has shown that maternal immune activation (mIA) is an important risk factor for neurodevelopmental disorders (NDDs) in the offspring. These disorders reduce the quality of life for patients and carers. They are poorly treated by existing medication and have a large economic cost burden. Both the pre and post-natal environments are critical for normal development of the fetus and offspring. Our multidisciplinary team has established a rat mIA model, work this application aims to continue. Evidence suggests that mIA, when accompanied by trauma in or around puberty, elevates the risk for later development of NDDs, particularly schizophrenia, above either risk in isolation. In this regard, the 'Second-Hit' appears critical for exaggeration of a pre-existing risk. For this reason we now want to extend our model to include a second hit. An animal model that translates to the human illness will enable new treatments to be developed.
What outputs do you think you will see at the end of this project?

Scientific publications, new information for people working to develop better treatments. A validated neurodevelopmental animal model to test new treatments.
Who or what will benefit from these outputs, and how?

Scientific publications, new information for people working to develop better treatments. A validated neurodevelopmental animal model to test new treatments.
The scientific community and general public, furthering knowledge, and improving understanding. The pharmaceutical industry, and drug discovery groups, as we will provide new treatment targets. We have close links with the pharmaceutical industry and will inform their drug discovery strategy through these links and via publication of our findings. Patients, carers and the NHS as ultimately this work will lead to new improved medicines for patients.
How will you look to maximise the outputs of this work?

We are committed to publication of positive and negative results, and to public engagement. We have already published our work refining the model and our methods. Several members of the team are experienced in public engagement.
This work started as a collaboration with a large pharmaceutical company, we will continue to collaborate with this sector. We will bring in more collaborators as the project evolves. The team currently consists of experts in behaviour, genetics, placental biology and development. We also engage with psychiatrist experts in this area.
Species and numbers of animals expected to be used

- Rats: 6000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rats are a popular choice for experimental work because of the detailed existing knowledge of their brain anatomy and behaviour. The rat has been chosen for the present work as much is already known about its cognitive behaviour and the brain functions controlling behaviour. We have extensive experience of studying behaviour in rats. All our current tests and protocols are validated for rats. Rats breed well in captivity and are well suited to longitudinal studies.
To allow us a better understanding of how schizophrenia develops from pre-birth to adulthood we have chosen to study the effects of mIA at critical stages of development. This is, pre-birth, young, adolescent and adult stages in male and female rats.

Typically, what will be done to an animal used in your project?

Pregnancy

Pregnant rats may be given a drug treatment regimen by mouth or by injection, or environmental treatment such as enrichment or exercise. This will be done before or after administration of an immune activating agent. A small blood sample will be taken from the tail vein at specified times after this to measure the immune response of the mother. The mothers will be checked regularly for behavioural and physical changes such as changes in grooming, general activity, body temperature and body weight caused by the infection. Some pregnant mothers will be humanely killed to allow collection and analysis of tissue from pregnant females and fetuses. Some pregnant females may be stressed by short term restraint at selected times during pregnancy.

Offspring

Following weaning, behaviour will be analysed in the offspring. The effect of treatments on offspring memory, behaviour and physiology will be investigated. The offspring may be given a drug treatment by mouth or injection, or environmental treatment such as enrichment or exercise. Some offspring will receive an anaesthetic to implant mini pumps for long-term administration of drugs. Some offspring will receive an anaesthetic to record brain activity while unconscious. These animals will be humanely killed at the end of the recording session without waking.
Some groups of offspring will be stressed by mixing up cage groups or short-term social isolation. At the end of the study, or as part of the experimental procedure the rats will be humanely killed.

What are the expected impacts and/or adverse effects for the animals during your project?

The administration of a maternal immune activator is likely to induce a mild and short-lived infection, slightly increased body temperature or mild sickness in the mother, lasting less than 24 hours. The treatments may cause mild short-term pain from the injection site and also cause mild and short-lived changes in behaviour such as increase/decrease in activity. Stress inducing techniques, such as restraint of the dam, introduction of a male in close proximity to the cage, may be used on some pregnant females and her pups which may cause short-lived, mild changes in behaviour, appearance and general well-being. Behavioural techniques, applied to the offspring, are generally not stressful and can, in certain cases, be considered enrichment for the animals. Some animals will be placed on mild food restriction during behavioural testing and lose no more than 10% of their free feeding body weight. We intend to stress some offspring in adolescence by mixing up cage groups or short-term social isolation. Some rats may undergo anaesthesia for surgery to implant drug delivery pumps under their skin. They recover from this procedure quickly and in most cases no untoward effects are observed. At the end of the study, or as part of the experimental procedure, rats will be killed humanely.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected maximum severity for both protocols in this license is moderate. Approximately 75% of the animals used will reach moderate severity and 25% of the animals will reach mild severity.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This work is complex and involves understanding the interactions between several body systems. In addition to the changes in the brain function of patients, these disorders are characterised by deficits in social behaviour, memory and mood. Such aspects of NDDs are not possible to model using cells or simulations. This work therefore must entail the use of animals as behaviour is a central feature of the project.
Which non-animal alternatives did you consider for use in this project?

None. However we did consider lower order animals such as drosophila.
Why were they not suitable?

Their behaviour and brain and life cycle is too far away from mammalian species to provide a translational model. On consultation with pharmaceutical company colleagues, they considered rodents the most suitable species for this work.
We did not consider non-animal alternatives because there are no non-animal models or systems that replicate the complex interactions and architecture of the central nervous system and the way it communicates with the immune system. If any relevant non-animal alternatives (or less sentient species that are suitable) become available during the course of the project, we will implement these in our studies. The animal studies will be accompanied by in depth analysis of brain and placenta tissue which may identify a biological marker that could allow us to develop subsequent isolated culture systems to study the pathology of these disorders.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This is based on data generated from similar studies under the same experimental conditions. We consulted a statistician who advised on the minimum number of animals required to give us the maximum statistical power.

What steps did you take during the experimental design phase to reduce the number of animals being

used in this project?

We have optimised our statistical analysis through work conducted on our previous licence. We will also use the NC3R's Experimental Design Assistant.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will follow efficient breeding protocols in consultation with experts, carry out pilot studies with small numbers of animals and always aim to share tissue with team members and other colleagues.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We are continually refining our behavioural procedures to enhance animal welfare (using species relevant tests, minimum food restriction, food rewards). When we started this project, we optimised our methodology through an extensive study in non-pregnant rats. Since then, we have refined our methods.

Why can’t you use animals that are less sentient?

It is not possible to mimic complex interactions outside a living organism. Furthermore, this work must entail the use of animals as behaviour is a central feature of the project. We are unable to measure complex behaviour patterns in immature life stages, animals that are less sentient or that have been terminally anaesthetised.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Specific on-going refinements include: reduced use of food restriction for the behavioural tests, increasing use of species-relevant tasks, implementing enrichment such as play pens and tummy tickling. We have also optimised our dosing regimens, handling and dosing techniques in collaboration with experts in rodent handling, including reduced restraint. Where animals will be subjected to potentially painful procedures, we will use suitable pain relief to minimise any pain and suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We consult with experts to implement improvements in animal welfare. We have access to the extensive library of NC3Rs resources which includes guidelines, practical information and themed hubs. Links to publications, other online resources, and video and training materials.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We are registered to the NC3Rs and subscribe to CRACK IT innovation platform and receive updates on advances through NC3Rs newsletters. We are working with our local NC3Rs manager, on implementing environmental enrichment using play pens.

Home Office

NON-TECHNICAL SUMMARY

37.Development of a new generation of AAV vectors to treat monogenetic disorders and acquired conditions

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

AAV, Gene therapy

Animal types	Life stages
Mice	adult, aged, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our research programmes focus on the development of potentially curative treatments for several monogenetic disorders and rare acquired diseases, with a largely unmet therapeutic need, using advances in gene therapy technology. Over the next 5 years our focus is on inherited and immune-mediated disorders, using new vector designs that enable therapeutic expression of transgenic protein in appropriate mouse models without toxicity.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

We have used our gene therapy approach for treatment of patients with blood and metabolic disorders, with some patients now up to four years post treatment. So far, we have observed that endogenous 24/7 expression of the missing or non-functional protein and have learnt that the expression and efficacy of our gene therapy approach in mice predicted human response, indicating that evaluation of any new vector in a mouse model is essential. We have also learnt that immune response to gene therapy (pre-existing and/or acquired) can hinder the efficacy of gene therapy and prevent re-dosing. Understanding the mechanisms underlying this immune response is of crucial importance for future success of the AAV-focused gene therapy.

What outputs do you think you will see at the end of this project?

We expect to demonstrate that gene therapy with AAV vectors will be safe and effective in the treatment of a range of monogenetic disorders, especially those that remain a significant burden on the healthcare and society. The new vectors being constructed for these disorders are likely to be more potent than has been the case to date. In addition, the quality of vector is improving over time. These advances reduce the risk of toxicity whilst reducing the amount of vector required for the effective gene transfer. The results we obtain with our new vectors will provide new information about the new therapeutic approaches for the explored diseases and will be published in scientific publications to disseminate the knowledge acquire to a wider scientific/clinical community.

Who or what will benefit from these outputs, and how?

Gene therapy has transformed the lives of patients without persistent or late toxicity to date with three approved AAV gene therapies and two for haemophilia submitted for approval. Single dose AAV gene therapies are anticipated to be durable resulting in health savings on protein/enzyme replacement therapies (~£150k to £350k per patient per annum for life for protein/enzyme replacement), ~£70k for surgeries such as kidney or liver transplants. Successful gene therapy can also have benefits for the whole society via reduction in economic and societal loss due to absence from work or school and

reduction in demand on hospital care and social services. The work proposed will help us develop novel gene therapy approaches for other disorders where current treatments are not optimal or not available.

How will you look to maximise the outputs of this work?

In our pursuit of the most appropriate methods for testing our vectors, we will form many international collaborations with key opinion leaders in the field, both academic and industrial to join efforts in our attempts to find the best AAV approach for various indications with not just therapeutic but curative potential. We will make sure our results, both positive and negative, will be published in peer-reviewed scientific publications and made available for the wider community.

Species and numbers of animals expected to be used

- Mice: 4500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the lowest vertebrate with well characterised disease models including genetic manipulation that is used to model human inherited diseases. No other species could fulfil the requirement of this programme, especially in term of generation of pre-clinical proof of concepts, to the same extent as the mouse. Most of our proposed protocols will use animals at the adult stage to ensure that all the physiological systems are functionally and fully developed. This is also relevant in terms of refinement, as we will avoid using juvenile or aged mice, without a valid scientific objective.

Typically, what will be done to an animal used in your project?

In a typical experiment, animals will be allowed a period of acclimatisation before any procedure is performed to reduce stress. Biological samples are collected before starting any treatments to have a benchmark data. The animals are then injected with a gene therapy vector and the biological samples are collected at regular intervals to monitor the effects of the gene therapy. The minimum duration of an experiment can be 4 weeks or up to 24 months in long-term experiments. At the end of the experiment, the animals are humanely killed and several samples and biological fluids are collected for extensive analysis. In the experiments focused on specific disease models, animals will be allowed a period of acclimatisation before any procedure is performed to reduce stress. Biological samples are collected before starting any treatments to have a benchmark data. The animals are then injected with a gene therapy vector and allowed a period of up to 4 weeks before the pre-clinical models of diseases are started. For example, changing the standard rodent diet to a diet that causes the animals to accumulate fat in the liver (a model of non-alcoholic steatohepatitis), or injecting or applying chemicals that cause local or systemic inflammation and tissue scarring (similar to a range of auto-immune diseases). During the progression of the disease models, biological samples may be collected at

regular intervals to monitor the disease progress. At the end of each respective pre-clinical model of disease, the animals are humanely killed and tissue samples, in addition to biological fluids are collected for extensive analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Most of our protocols and procedures are not expected to cause adverse effects that are long-lasting. However, animals can experience discomfort and pain from the injections and/or sample collections. We will monitor these and provide pain-relief as needed. Other effects of treatments that we will monitor include changes in body weight and behaviour and general overall condition. We anticipate that adverse events will be transient (1-6 hours) and any animal showing adverse events that do not improve within will be humanely killed. In the experiments focused on specific disease models, we have proposed the most refined disease models that result in similar clinical outcomes that are observed in humans. Most of the disease models are expected to cause mild-moderate adverse effects as the disease models progress. This can typically cause modest weight loss which will be an important indicator of the welfare of the animal. We will monitor the animals frequently and provide appetising gel diet to counter the weight loss. If this does not cause an improvement, animals will be humanely killed. We have established clear endpoints in all models that do not cause unnecessary discomfort and pain to the animals, yet will allow us to address our scientific questions.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The degree of severity is expected to be moderate or less depending on the procedures we propose to use. All of our protocols will be performed on mice. Mice will be closely monitored for development of any adverse clinical signs and clear endpoints are defined in the protocols in order to minimise conditions of distress. At the end of each experiment and at well specified humane endpoints, mice will be humanely killed, and blood and tissues will be collected for analyses.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

All of our proposed work using animals will be preceded by studies using mouse/human cell lines including stem cells, and organoids (miniature organ-like 3D cluster of cells), co-culture and organ-on-chip cellular assays which are becoming increasingly important in modelling different disease

conditions. These crucial studies will allow us to select the appropriate gene therapy vectors for evaluation in mice.

The core physiological systems of the body, including the immune system, are controlled by complicated inter-dependent mechanisms that are not replicated by cells grown in a laboratory. The mouse represents the lowest vertebrate species that is accessible to genetic manipulation for the understanding of mechanisms of diseases, penetration of liver expressed therapeutic proteins into deep tissues associated with disease and disease substrate clearance/pathology resolution, transport and protein targeting mechanisms. Additionally, mouse and human genes are similar by approximately 80%, which would allow us to predict the therapeutic effects of our gene therapy vectors in human patients. There is a large amount of published scientific body of work that shows that our proposed models have a good degree of translation to the effects observed in humans. Importantly, all of the procedures that will be undertaken have been refined to minimise suffering.

Which non-animal alternatives did you consider for use in this project?

1. Cell culture-based approaches - Where possible the gene therapy vectors we develop are assessed during in vitro assays using commercially available mouse and human cell lines to ensure efficacy. This is employed for all our approaches and for each new batch of vector.

2. We are developing and evaluating disease specific human cell models obtained from stem cells to determine if these can replace animal models for evaluation of gene therapy vectors.

3. We are evaluating human liver cells grown in 3D cultures (organoids) to see if these can substitute mice for assessment of gene therapy vectors.

4. We are currently evaluating ‘organ-on-chips’ assays that use human cells to model a physiological system (e.g., the liver) and which can be manipulated to mimic clinical conditions (e.g., fatty liver disease). This will allow us to assess the efficacy of our candidate vectors in the therapeutic indication before commencing animal studies.

Why were they not suitable?

Murine models of human disease serve as an important tool for establishing pre-clinical proof-of-concept, and for assessing the efficacy and safety profile and immunological consequences of our gene therapy approaches. Based on the clinical data becoming available from clinical trials, it is becoming increasingly important to understand the effect of the host immune response on the efficiency of gene transfer after gene therapy treatment. The body’s response to gene therapy vectors involves multiple systems, organs and cell types. Additionally, the complexity of the immune response cannot be assessed in in-vitro settings. The use of in vivo models is essential to refine existing therapies, and develop new therapies, as they will allow us to detect the persistence of the gene therapy, their efficacy in modifying/preventing diseases, and any potential toxicity. In vitro systems, while useful, do not fully replicate the complexity of immune interactions or disease mechanisms in vivo and it is essential to use appropriate and robust animal models to understand these processes.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimation of the numbers of animals intended to be used under this PPL has been agreed with project leads at our company and aligned with our strategic goals for the next 5 years. Additionally, we have also used our experience from our previous project licence to estimate the number of animals that we will use.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used Power calculations using the NC3R's Experimental Design Assistant and statistical consultants to reduce the number of animals being used whilst ensuring that sufficient data is obtained to answer the research question. Additionally, we have designed longitudinal pharmacokinetic/pharmacodynamic experiments that will allow us to obtain data from the same animal at different time-points, instead of using different animals for the distinct time-points. These experiments will be performed at the highest standard that will not compromise the welfare of the animals and will also allow us to significantly reduce the numbers of animals being used in this project. Similarly, we designed experiments that will allow us to maximise the information obtained from each animal via advanced molecular biological assays on post-mortem tissue/biological samples following the completion of in vivo experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In addition to good experimental design, we will extensively use computer modelling and cell-based evaluations to reduce the number of animals used in our project. We will also assess sharing tissue samples from different experiments to address specific objectives, if feasible. We will consult with the experienced animal care support staff at our animal facility to implement strategies that will allow us to maintain efficient breeding of animals. This may involve freezing of sperm and embryos to reduce the number of animals kept alive for specific disease models. Importantly, our study designs will be reviewed by statisticians to ensure that we design experiments that will use the minimum number of animals to achieve our objectives.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The majority of our animal models are genetic or induced to resemble human disease conditions. For example, we will use mice that are genetically modified to model inherited human conditions (for example inherited metabolic disorders). These mice do not have the full clinical phenotype seen in human patients but have the same dysfunction (i.e. disease substrate build up), which allowed us to use this model to successfully develop a treatment for patients. We are now proposing the use of additional pre-clinical models of chronic diseases using models that are known as gold standard. These models closely resemble the human patients with the least suffering and distress caused to the animal. We will design experiments that will allow us to determine the efficacy of our candidate gene therapy vectors in improving these conditions by injecting the gene therapy vector by different routes. The number and volumes of administrations and blood samples will be minimised while ensuring scientific validity and minimising discomfort. Before any experiment is performed, animals will be acclimatised to procedures.
Environmental enrichment will be provided in home cages.

Why can’t you use animals that are less sentient?

We have chosen to work with mice as they are the lowest vertebrate group with well characterised disease models. The mouse models we propose to use in this project license are already well established, characterised and described in the scientific literature.

As described before, unfortunately, in vitro studies do not completely predict outcomes in humans. For example, a common gene therapy vector (AAV8) performed poorly in vitro but were highly efficient in animal models including mice. Efficacy in mice predicted the outcomes in human patients with inherited bleeding disorders (haemophilia B). Importantly, the mouse immune system is the best characterised amongst vertebrates and there is a strong degree of similarity between aspects of the human and mouse immune systems. Most reagents and tools required for the proposed plan of work have been designed for use in mouse models and for the detection and tracking of murine immune cells. Immunological assessments are going to be an important part of the studies we will perform over the next five years.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All the protocols have well defined end points and mice will be humanely killed with an appropriate Schedule 1 or non-schedule 1 methods within 12 months of receiving gene therapy vector or earlier where indicated. In some studies, an extended duration may be required (16-24 months). Full effort will be made to ensure animal well-being and comfort, and to minimise pain and distress. Good handling will minimise discomfort of the animal during the procedure. Sites of injection will be monitored carefully to prevent wound infection. If needed, we will apply local analgesics over the affected region to improve recovery. Aseptic techniques will be used at all times. Experimental animals will be monitored at least daily by our research group and staff within the animal unit. Details of animal experiments in progress will be shared between our research team and the staff within the animal unit to ensure that unexpected adverse events are quickly resolved to minimise harms to the animal.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will use the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines in addition to Home Office published guidelines (e.g., Guidance on the Operation of the Animals (Scientific Procedures) Act 1986) and relevant LASA guidelines. Additionally, all murine models that we propose to use have extensive literature resources available which will allow us to conduct our experiments in the most refined way.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We currently receive news bulletins and invitations to specialist seminars organised by the NC3Rs and our animal facility is very engaged with the NC3Rs community. We will maintain this engagement to stay informed about the advances in the 3Rs, in addition to staying up-to-date with scientific publications that highlight 3Rs principles. We will implement these advances effectively by working with the animal facility technical staff and our research team.

Home Office

NON-TECHNICAL SUMMARY

38.Development of Treatments for Degenerative and Traumatic Retinal Disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Exosomes, Glaucoma, Optic nerve injury, stem cells, neuroprotection

Animal types	Life stages
Rats	adult, aged
Mice	aged, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to test multiple therapeutic agents as treatments for blinding diseases of the retina. These agents include stem cells, exosomes/extracellular vesicles, and genetic compounds known as miRNA. We also aim to develop animal-free models of retinal disease that are comparable to those currently used in eye research.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Vision is the most important sense for humans, and it is required for individuals to maintain their independence in today's society. Vision loss is a devastating condition and is associated with some of the lowest quality of life scores. Many diseases that lead to vision loss are age-related and are thus becoming an increasing problem in a society whose life expectancy increases. For example, glaucoma
affects 80 million individuals and is estimated to affect 120 million by 2040. In this example, no treatment exists that directly prevents the retina from degenerating. This proposed project seeks to develop the first novel treatment to directly protect the retina and prevent visual decline. In the development of treatments, it is also important to consider the models utilized. There are huge differences between human and animal retina, which although do not invalidate the use of animals in eye research, do create a need for good human in vitro models. As part of this project, human retina generated from human embryonic stem cell lines will be injured in a variety of ways in an attempt to recapitulate human disease and therefore act as an animal-free replacement to eye research.

What outputs do you think you will see at the end of this project?

This project will generate a number of outputs.
Firstly, the work described in this project is covered under a patent and we seek to generate the first neuroprotective treatment for patients with glaucoma and other retinal diseases, preventing visual decline and blindness.
Secondly, publications describing exosomes and their mechanisms of action will continue to be published, in an effort to aid other research groups assessing their clinical efficacy in a range of other diseases including spinal cord injury, stroke, and Alzheimer's disease.
Finally, the project will generate data integral to obtaining further grant funding which will help see the above two outputs realized.

Who or what will benefit from these outputs, and how?

Firstly, this project benefits the vesicle/exosome research community as it seeks to understand the importance of exosomes, their role in homeostasis as well as their therapeutic potential as well as the potential for the presence of distinct
subtypes. For example, the exosome research committee at the National Institutes of Health is a collection of researchers working within this field. Equally multiple researchers in UK institutes all lead research groups focusing on exosomes/microvesicles and will directly benefit from the data derived in this project. Many groups have begun working within the field of exosome subtypes and part of this proposal seeks to add to this field, understanding not just the differences between each subtype but also how this effects their therapeutic efficacy. We will also conduct miRNA next generation sequencing (RO4) whereby data will be uploaded and

made available to researchers*.
The second beneficiary is the neuroscience community and in particular, the neuroprotection, axon regeneration and, eye field. The importance of this research to the field is exemplified by the current Audacious Goals Initiative (AGI) set out by
the National Institutes of Health, National Eye Institute which is "tackling the most devastating and difficult-to-treat eye
disease". Institutes all across the United Kingdom have many researchers actively engaged in this research field. Collaborations between this proposal and their own research projects as data is accrued provide mutual benefit.
Finally, one of our aims is to develop a human in vitro model of optic nerve injury (and test exosome treatments in this model), using genetically modified human embryonic stem cells. Many research groups are utilizing these cells as they have been made widely available and all data published helps researchers understand these cells and their strengths/limitations as a human model of retinal disease
. By developing a model of eye disease that is animal free, it also seeks to push the field away from the use of animals. This will greatly benefit animal welfare as less animals will be used in eye research without detriment to research output.
*All results will be published in high impact open-access journals. Published manuscripts will be placed into the home establishment's dedicated repository. After the required timeframe (6 months STEM/12 months SSAH) and based upon the publisher's rules, articles will be made open access as part of the Green Open Access Route. If the publisher's rules prohibit the Green
Open Access Route, the home establishment will pay Article Processing Charges and publish in the Gold Open Access Route.
Embargo times will be checked to be compliant with the journal and MRC, and the University will automatically make the
article available after the embargo. Bibliographic metadata will be made accessible with author set restrictions to maintain any confidentiality requested. The information will be added to the Open Archives Initiative Protocol for Metadata
harvesting (OAI-PMH) under the keyword "MRC" and indexed into frequently used search engines such as Web of
Knowledge and Google Scholar. Metadata will also link to the Universities Published Data Archive where data produced during this fellowship with be published in accordance with Open Data requirements. Data will be managed continuously to ensure it maintains a high standard with emphasis on clarity and will abide by the relevant institutional and departmental policies.

Impact Summary
Currently, no neuroprotective treatment
exists for glaucoma. Visual deficits have serious consequences to patient well-being and health (>80m individuals) and a loss of vision is associated with some of the lowest quality of life scores. This project is of particular importance as it focuses on "healthy ageing", of which glaucoma, an irreversible condition, can be considered the prototypical ageing disease. Finally, the pro-regenerative aspects of this project stand to not only benefit the eye but other central nervous system disorders such as spinal cord injury.
This project seeks to benefit those patients with glaucoma and other similar eye disorders as well as the ageing population of which a significant portion will develop such diseases.
Commercial beneficiaries are based on ensuring my research is patented and exploited. The work discussed in this
proposal is covered under a patent for which I am listed as the inventor. Future discoveries that come about from this proposal will be effectively exploited in the same manner. Knowledge and IP management will be based on the requirements defined in the official documentation as well as the Grant Agreement. University teams are available to provide support regarding the exploitation of IP and will maintain dialogue with my research group to ensure IP is protected. They will continually monitor the nature and size of commercial

markets to determine possibilities for exploitation via identifying potential licensees, customers, partners and collaborators. The team has experience and provides support for proof of concept, creation of spin-off companies, venture development and start-up incubation.
With regards to training, funded post-doctoral researchers (as well as potential PhD/MSc/summer students) will gain a highly interdisciplinary background, being trained in neuroscience, molecular biology, vesicle biology and bioinformatics.
They will be trained to utilize the most cutting-edge in vitro and in vivo techniques as well having the opportunity to gain supervisory skills (MSc students will be joint supervised by myself and the post-doc). Students will attend training sessions held by the University, such as the post-doc development centre which provides skills in communication, organisation, grant/paper writing and presentations. The post-doc will also attend international conferences and present their findings to world leaders in the field.

How will you look to maximise the outputs of this work?

As described above, all publications will be made open access and annual conferences will be attended to ensure data is disseminated in an open and timely fashion. We will maintain strong collaborations with the National Institutes of Health, the largest research institute in the world, and continue to work together publishing papers and applying for patents. We will work closely with the commercialization teams, as discussed above, to ensure the maximal impact can be achieved. Unsuccessful/non-significant results will be made available, either as an inclusion in published manuscripts as supplemental data, or being uploaded to research communities such as ResearchGate.

Species and numbers of animals expected to be used

- Rats: 500 per 5 year
- Mice: 80 per 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

To study eye research, an animal is required whose visual system best represents that of a human. It is also important that the animal is an adult, as the adult visual system is distinct from that of a young animal whose regenerative potential is greater. Since glaucoma and other ocular diseases occur predominately in adults and often in the aged population, this must be represented in the animal model. Between mice and rats, rats are the optimal choice for several reasons. Firstly, rats are larger which makes the surgery easier and reduces complications. Injections into the eye can be delicate and this is made easier with the larger eye of the rat, particularly when injected into the anterior chamber.
Secondly, the injury response to nervous system injury is more similar to humans in rats than mice.
Finally, in glaucoma, damage occurs in a particular structure of the eye known as the lamina cribrosa.
While this structure is present in humans and rats, it is not present in mice. For these reasons, rat models of glaucoma and traumatic optic neuropathy are the most ideal. The mouse DBA/2J model is however a long-term model and does have the advantages of testing the long-term effects of candidate treatments.

Typically, what will be done to an animal used in your project?

With regards to glaucoma, animals will receive injections into the front chamber of the eye, either beads or cytokines, which will induce an elevation in eye pressure and thus, glaucoma. Pressure is monitored using a painless rebound tonometer. Treatments are delivered into the eye similarly by intraocular injection, identical to how many other treatments are given to patients with eye disease. Ocular injections as well as ocular pressure recording require the animal to be anaesthetised, not to minimize pain as none is expected, but to prevent the animal moving during the delicate injection.
The second model is optic neuropathy. This surgery requires a small incision on the head, identification of the optic nerve that runs out the back of the eye, and the crushing of said optic nerve using forceps/tweezers. The wound is sutured afterwards, and animals recover completely in 1-2 days. Analgesic is given during these 1-2 days of recovery.
All animal models are expected to last 1-2 months and the surgery is only done once at the beginning of the procedure. Treatments are given either once a week, or once a month depending on the efficacy. Visual assessments (imaging) are also done (once a week/month) and are painless but require anaesthesia to immobilize the animal.
The DBA/2J mouse model is a strain of mouse that develops ocular hypertension without intervention and is kept for 9 months from age 3 to 12 months. Injections, described above, are delivered once a month.

What are the expected impacts and/or adverse effects for the animals during your project?

The induction of elevated intraocular pressure is used to model glaucoma and although visual decline is expected in animals, few adverse effects are expected. Rodents are not dependent on their vision and thus their behaviour is not affected by this perturbation. The most common adverse effect is dry eyes which is treated easily with eye lubrication drops. Other less common side effects such as pain and irritation of the eye, and inflammation are possible and are a consequence of infection resulting from the injection. These are detailed in the score sheet and their management may include anti-septic eye cream/ointment or schedule 1, depending on the severity.
Optic neuropathy involves a head wound and thus, pain and discomfort is expected for the first 2 days of recovery. An expected adverse effect is wound reopening which can occur if rats irritate the surgical site (scratching or cage-mates biting). Analgesia is provided to animals during these recovery days and the pain and discomfort is managed according to the score sheet.
In the DBA/2J mouse strain, development of cataracts is common and is typically permanent but not painful. Aged animals (10+ months) of this particular strain are also susceptible to cardiac calcification and tend to die sooner than other mouse strains. Between 10-12 months of age, which is an important step in this project as this is when the majority of visual decline will occur; approximately 10% of animals will not recover from anaesthesia during pressure recordings due to the above mentioned cardiac calcification.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected category for all animals associated with this procedure is moderate severity (100%).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The eye is a complex tissue with multiple interconnected cell types. To ascertain that our candidate therapies would have a clinical benefit on patients, multiple tests are required that would not be possible on anything but an in vivo system. These include live imaging of the retinal morphology overtime using optical coherence tomography, and live recordings of the electrical function of the retina in response to light using electroretinography. Both of these techniques are used in the diagnosis process in patients as well as to monitor disease and treatment progression. We also wish to understand the longevity of this treatment when it is administered into the eye, how often the treatment must be re-administered and any interactions with the rest of the body *via* the systemic circulation. All of these require the use of an in vivo system.
Given the novelty of exosomes/extracellular vesicles as a signalling mechanism, it is not currently understood what role they play in maintaining tissue homeostasis. Homeostasis is complex involving signals between multiple cell types and, by definition, involves the tissue acting in its normal environment which necessitates the use of animals.

Which non-animal alternatives did you consider for use in this project?

My work utilizes two non-animal alternatives, both of which are considered (as well as replace certain elements of this project).
Partial Replacement - Rodents are sacrificed and the eyes/retinae are removed and cultured. Since the optic nerve is severed, these cells will die in culture in a way which recapitulates the death seen after optic nerve injury *in vivo*. Treatments can be added to the culture medium and neuroprotective and neuritogenic effects can be measured.
Total Replacement - Human Embryonic Stem Cell lines are differentiated into retinal cells using a well published 35-day small molecule regime. Unlike in the above model, retinal cells do not die in culture so a microtubule poison (colchicine) is added to instigate retinal degeneration. Treatments can be added to the culture medium and neuroprotective and neuritogenic effects can be measured.

Why were they not suitable?

Rodent retinal cultures do not full recapitulate the complexity of retinal disease, and are more associated with traumatic injury as opposed to the slow progressive degeneration seen in glaucoma. Retinal cultures are only viable for 3-4 days and so while they are very useful for providing hints as to which therapies may have clinical efficacy, they do not serve as a suitable replacement for *in vivo* models.
Human retinal cultures are a strong contender for the replacement of animals however they are held back by a lack of good methods on how to injure them in a way which fully recapitulates the disease seen *in vivo.* Since they do not degenerate spontaneously in culture like rodent retina, degeneration must be induced externally. One particular project within this licence is to develop this model further, testing new injury systems to better model human disease (and comparing them to current regulated procedures and established treatments). As of now however, the system serves as a useful approach to ensure that therapies are applicable to human retina, but cannot as of yet model the complex disease in full.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The models used in this proposal are those I have used routinely in the last 8 years and thus, a large amount of data is available to aid in the estimation of animal numbers. Indeed, one of my open-access publications focused entirely on study design and power calculation-informed decisions on the number of animals required to determine varying effect sizes in eye research. These power calculations are available to all eye researchers utilising these models and have been used in the estimations in this proposal. Briefly, the rat model of optic nerve crush is incredibly reliable and power calculations (alpha value = 0.05, Beta value = 0.2) determine that 5 animals per group are required to detect a difference in retinal cell numbers by 20%. Regarding glaucoma models, standard deviations are higher and thus 7 animals per group are required for the same variables. One additional animal than that identified via power calculation is utilised. Thus, for every treatment measured, 6 animals for optic nerve crush and 16 animals for glaucoma (2 different models) are used. While it is not currently known how many treatments groups we will utilise as more candidates will inevitably be discovered, we have currently identified 8-10 treatments which would require 220 animals as part of objective 2. Some groups will require 5 extra animals which will provide the tissues necessary for sequencing as part of Objective 4. Objective 3 will aim to recycle data from animals used above but it will likely be necessary that some groups are specifically set up to derive data for Objective 3. A further 60 animals are required to address Objective 1 of this proposal which involves mainly retinal sampling before or after treatment. The numbers are based on the power calculation above but the only treatments used for further analysis are those that show the greatest promise in Objective 2.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To reduce the number of animals used we employ retinal cultures (described in the replacement section) to confirm therapeutic potential in our drug candidates prior to the move to *in vivo* testing. If certain exosome/extracellular vesicle formulations are ineffective at promoting any therapeutic efficacy *in vitro*, they are excluded from the *in vivo* study. We also generate as much data as possible from the same animal cohort. A single animal can be live imaged, functionally and behaviourally tested as well as histologically analysed post-mortem. By incorporating all of these tests from the onset, we do not need to use more animals in the future to test the same treatment. When using histology, the retina can be taken as a whole-mount or as tissue sections. While the retinal whole-mount is considered more reliable, tissue sections reduce animal numbers as a variety of analyses can be done on the 100+ sections that can be collected per eye. Our previous publication has demonstrated that retinal sections are as reliable as whole-mounts and thus, using sections, animal numbers will be reduced significantly.
The experiment is planned on the basis that both males and females will be used, within the same study. This will allow us to compare any sex-specific effects while not reducing the reliability of the overall study as we can use a statistical blocking approach on the collected data.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

While the number of rats per group cannot be reduced further, we can reduce the number of animals used in *in vitro* tissue culture experiments. As the culture experiments require eyes, we will collaborate with other research groups working on brain tissue. Coordinating with their group can mean that the same animal can donate tissue for both studies.

With regards to the development of a human *in vitro* model retina disease (objective 3), its reliability needs to be compared to the currently used rodent models. While as a stand alone project this would mean animals are required to test these models and compare data to the developed *in vitro* systems, by joining the two project aims, significant reductions in animal numbers can be achieved. As an example, testing three formulations of exosomes in a model of glaucoma will also include a 4th control group where no treatment is added. This very same data will be compared to the developing *in vitro* models, assessing their reliability. Thus, the same animals will address two distinct project aims.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The models detailed in this project licence application all seek to reduce vision in animals.
Distress/suffering - removing an animal's primary sense can be considered distressing to said animal and indeed the basis of this application is to prevent and treat vision loss in human, which is associated with significant loss of quality of life. However, rodents, unlike we humans, do not depend on vision. A rat's vision is estimated to be 20/600 (20/20 is normal healthy vision in humans) with an albino rat going down to 20/1200. It is worth noting a vision of 20/200 is considered legally blind. It is not possible to discriminate between control animals and animals subjected to the models described in this licence as their behaviours are identical. Considering that only one eye is affected in these models, it can thus be strongly argued that animals do not suffer any lasting distress/harm.
Pain - The glaucoma models typically involve ocular injections under anaesthesia. Note that these are routinely done in humans (without general anaesthesia) and thus are known to not cause any lasting pain any more than an intravenous or intraperitoneal injection. The optic nerve crush model utilises an intra-orbital approach and thus require an incision on the scalp as well as a small incision through the anterior portion of temporalis (jaw muscle) and mobilisation of the harderian gland. It is thus expected that following recovery, the animal will be in some mild pain for 1-2 days. This is adequately managed with analgesics and animals return to normal behaviour within 24 hours. These models have been developed over several decades to produce the least suffering in animals and are routinely used as the gold-standard in eye research.
DBA/2J mice are more susceptible cardiac calcification as they age and subsequently not recovering from anaesthesia (approximately 10% of mice). This effect becomes significantly more pronounced past 12 months and hence, stopping the experiment at 12 months prevent much of the detrimental effects while also allowing the acquisition of usable data.

Why can’t you use animals that are less sentient?

The central nervous system has evolved measures to prevent repair and regeneration, and these measures are not present in lower order animals. For example, injury to the visual system of a zebrafish is temporary and the eye and optic nerve completely repair over several days. Thus, testing treatments in these models becomes more difficult, as does trying to elevate pressure in their eye due to distinct differences in their physiology. Lower order life forms such as insects lack the complex eye structures seen in humans and again, glaucomatous damage of the optic nerve cannot be modelled.
There are distinct differences between rats and mice that make rats the more suitable choice. Firstly, given injections are being given into the eye, or surgery is being done around the eye, the increased size of the rat is hugely beneficial to surgical success and well-being of the animal. When injecting the rat's eye, avoiding the large lens is a requirement to ensure the results are viable. This potential complication is rarely seen when using rats compared to mice. In glaucoma, damage to the optic nerve occurs in a structure called the lamina cribrosa, an area just behind the eye. This structure is present in the rat but not in the mouse. Finally, the scarring and fibrotic response that occurs after central nervous system injury is distinct in rats and mice, and is more similar to the human process in rats than mice.
One benefit to using mice is the genetic models and strains available. One such strain is a DBA/2J mouse that spontaneously generates elevated intraocular pressure and glaucoma. Despite the disadvantages discussed above, the one benefit to this model/strain is that it occurs without intervention and is a 12 month process. This extended timescale is more similar to the human condition than the models used in rats and thus is useful for ensuring therapies are still viable over long periods of time.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Optic Nerve Crush - Animals will receive increased monitoring for the first few days following the procedure to ensure animals are not experiencing any pain and the wound heals effectively. VetBond glue (or other adhesive) as opposed to sutures will be strongly considered for wound sealing as we have demonstrated that recovery is faster and wound reopening is less likely. It can be argued that it is more uncomfortable for the animals so this will also need to be considered. Animals tend to open each other's head wounds when sutures are used, which then occasionally requires isolation of said animal. VetBond ensure animals can be housed together for the entirety of the project and will be mandatory if wound re-opening occurs to a greater frequency than detailed in our adverse effects. Only one optic nerve will be crushed so animals will retain their vision.
Glaucoma - The induction of glaucoma is done through the intraocular injection of substances and thus, refinements below are equally valid here. To monitor the pressure, a Tonometer must be used.
Typically, animals are anaesthetised to prevent variable stress reactions from influencing the pressure reading, since the animal must comfortably sit still as the rebound tonometer gently touches the eye. We will however consider training the animal the sit still for the tonometer recording, and some labs have had success in this regard.
Intravitreal injection - Ocular injections are a simple procedure but due to the delicacy of the injection site, anaesthesia is still required. Adequate training will ensure that the duration of anaesthesia is kept to a minimum. An efficiently training surgeon can perform the injection in under 5 minutes, including anaesthetic induction. Regular needles are too wide and thus cause excessive damage to the eye. We instead will use pulled glass capillary rods typically used to patch clamping cells, or Hamilton syringes.
Micro-needles are narrower than any commercially available needle and thus minimally damage the eye, allowing rapid healing.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Regarding eye research, we will follow the ARVO (Association for Research in Vision and Ophthalmology) guidelines for the use of animals in research. We will also adhere to the ARRIVE guidelines.

https://www.arvo.org/About/policies/statement-for-the-use-of-animals-in-ophthalmic-and-visionresearch/ https://www.nc3rs.org.uk/arrive-guidelines

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

A significant part of this project is the development of a human in vitro model of retinal disease, and thus, the 3Rs are intrinsic to this project. We will constantly engage with other eye researchers as well as the large amount of information available on the NC3Rs website. The project will incorporate new refinements as and when they are developed. We also hope that through the development of a in vitro model, certain aspects of the project can be replaced by these animal-free techniques.

Home Office

NON-TECHNICAL SUMMARY

39. Development, functions and programming of embryonic and adult immune cells

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project investigates (i) the **development and functions** of embryonic and adult immune cells as well as (ii) the effects of **early life adversity** on immune development and function and the consequences of this **"programming"** on later life health and disease.

A retrospective assessment of these aims will be due by 03 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

We currently have a **limited understanding** of what functions embryonic immune cells exert during gestation. Likewise, it remains controversial if their functions are distinct from those of immune cells generated at later stages. This is of particular interest for conditions where embryo-derived cells persist and co-exist with adult immune cells, like female reproductive organs and tumours. Similarly, we do not know if and how adverse early life environments change the normal developmental sequence and functions of immune cells, and if this predisposes for later life disease. This work is important to fill these **critical gaps**. In the future, it might help stratify individuals at risk for chronic inflammatory diseases like rheumatoid arthritis, and identify new avenues for preventive or early therapeutic intervention. It might also guide novel therapeutic approaches for pregnancy-related complications such as preterm birth as well as tumour immuno-therapy. Although fundamental in nature, this project thus has immediate **translational relevance**.

What outputs do you think you will see at the end of this project?

This project will primarily generate **new information** related to several scientific questions that are fundamental in nature, but nonetheless of immediate biomedical relevance: Our work will further improve our understanding of how the immune system normally develops, what functions the very first immune cells have in the embryo and in pregnancy, and if these functions are "hijacked" in tumours. We will also generate new information concerning the role of immune cells in maternal tissue remodelling in the context of pregnancy. Lastly, we hope to generate new insights in how adversity experienced early in life changes the normal course of immune development, and if and how this in

later life increases the susceptibility to tumours and chronic inflammatory disease like rheumatoid arthritis. We thus hope to provide a mechanistic understanding of cause-consequence relationships underlying observations made in human epidemiological data. These findings will be **published** in scientific journals, but may also be disseminated to the general public. Moreover, while the project will not directly generate products for therapeutic use, it might inform **new strategies** for medical purposes.

Who or what will benefit from these outputs, and how?

In the first instance, this project will benefit the **scientific community** by filling critical gaps in our understanding of immune development. It will also benefit the research field by generating new models with which to address questions that have remained unanswered or controversial. It might also benefit **society** by increasing **awareness** of how early life adversities can have long-lasting impact on the immune system. In the **future**, this work might also help **meet clinical needs**. Specifically, it might aid stratification of individuals at risk for chronic inflammatory diseases like rheumatoid arthritis, and identify new avenues for preventive or early therapeutic intervention. It might also guide novel therapeutic approaches for pregnancy-related complications such as preterm birth, as well as tumour immunotherapy.

How will you look to maximise the outputs of this work?

We aim to maximise the output of this project in several ways:

- This work will be highly **collaborative**. It will involve existing and new collaborations, both locally and internationally, and regular exchange on technical aspects and progress of the project.
- We plan to **disseminate** the data and knowledge generated within the **research community** through participation in expert **congresses** in relevant fields (e.g. Immunology, Developmental and Reproductive Biology, Rheumatology), **seminars** at other research institutions and **publications** in peer-reviewed journals. We also plan on disseminating our findings to the larger public through in science communication efforts and **public outreach** activities. These are strongly supported by our Centre and the University. We also intend to share within the scientific community any **unsuccessful approaches**, with the aims of troubleshooting these and preventing unnecessary unknowing duplication efforts. Where possible will we also publish negative data in scientific journals. Although unfortunately rather uncommon, there has been a recent surge in publications reporting the absence of phenotypes in studies aimed at understanding mast cell functions, or studies refuting functions previously attributed to these cells using different models. This example illustrates that the field is growing increasingly susceptible for sharing negative findings.
- Where appropriate, we also aim to interact with and share our efforts and findings with more specific public bodies such as **patient initiatives** (e.g. for rheumatoid arthritis) and **charities.**

Species and numbers of animals expected to be used

- Mice: 12.000 + 30.000 additional offspring

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Central questions underlying this project are how the immune system develops, and what its functions are at different life stages. Immune development is initiated early in gestation. We are therefore interested in animals across the entire lifespan, ranging from embryos to newborn, juvenile and adult animals as well as pregnant females. In addition to our interest in embryos that necessitates pregnant adults, we are also interested in them in their own right where the role of maternal immune cells in pregnancy-related tissue remodelling are concerned.

While we are using and analysing animals of all life stages, experimental procedures will only be directly performed on adult animals, with very few exceptions in which substances or cells are delivered directly to embryos or newborn mice.

Typically, what will be done to an animal used in your project?

Experiments are aimed at studying either (1) the origins and functions of immune cells in development and pregnancy or (2) the effects of prenatal adversity on immune development and disease susceptibility.

(1) Origins and functions of foetal or maternal immune cells in development and pregnancy. (1.1) Successfully mated female mice will proceed through pregnancy with or without an inflammatory challenge. Where inflammation is induced, this models acute inflammatory episodes or infections and in most cases will induce partial loss of foetuses or preterm birth. Inflammatory agents will be delivered typically by injection or orally. (1.2) Pregnant mice will undergo usually one additional treatment aimed at modulation of inflammation, the immune response or gene expression and again consisting of one or several injections or oral deliveries. Exceptionally, this will be replaced by so-called "minipumps" that deliver substances more continuously and that are surgically implanted, usually before pregnancy. In some cases, blood will be drawn on up to two occasions. Other experimental readouts will be non-invasive and include ultrasound imaging and monitoring by cameras. (1.3) Animals will be terminated without additional treatments and tissues analysed either before or after giving birth.

(2) Effects of prenatal adversity on immune development and disease susceptibility. (2.1) To generate mice that have prenatally experienced adversity, inflammation or arthritis will be induced during pregnancy. Where inflammation is induced, these models chronic low-grade inflammation and does not normally affect pregnancy success. To do so, substances will be delivered to pregnant mice either orally or by injection, typically on one or several occasions at different stages of pregnancy. In some instances, embryos will be injected directly using an ultrasound-guided method. In addition to injections, blood will be drawn from some pregnant mice in order to measure inflammation and disease parameters. A very limited number of mice will undergo surgery before pregnancy, with the aim of placing pumps that will continuously deliver substances to these animals. (2.2) The offspring of these pregnancies will be allowed to grow adult. (2.3) These offspring mice will then be subjected to treatments that either label immune cells according to their developmental origins, induce tumour growth or arthritis as well as additional treatments that modulate the immune response, disease or

gene expression. Typically, animals will undergo maximally two procedures that consist of one or several injections. In a small minority of mice, surgery will be performed instead of injections to place minipumps that deliver substances continuously. In addition, blood will be analysed from some of these animals. In most cases, additional analyses will be non-invasive, such as clinical scoring of joint swelling or tumour size. (2.4) Experiments will be terminated and tissue analysed at different disease stages.

What are the expected impacts and/or adverse effects for the animals during your project?

Adverse effects on animals may be caused either by genetic modifications or by experimental procedures and disease models.

Genetic effects: We will be using genetically modified animals, some of which may show preterm birth or foetal death during pregnancy. Although we are also using animals that are genetically predisposed to developing diabetes, we will normally only use these prior to disease onset for breeding.

Experimental effects:

- **Substance administration**: The nature and delivery regimens of most substances administered in this Project are not expected to cause persistent adverse effects. In rare exceptions, irritation may occur. Some substances used to modify gene expression may interfere with the ability of pregnant animals to give birth. In such cases, mothers will be humanely terminated, their pups delivered and transferred to foster mothers.

- **Surgical complications**: Surgical and post-surgical complications such as infections are extremely uncommon. Nonetheless, we strive to use refined methods circumventing the need for surgery and expect to perform only a very restricted number of surgeries.

Disease models:

- **Acute inflammation**: will be induced in pregnant animals at different time points of gestation. While inflammation may be accompanied by symptoms of discomfort, these are usually of only transient nature.

- **Rheumatoid arthritis**: is a chronic inflammatory disease characterised by joint swelling. Further symptoms may include weakness, loss of body weight, lameness and a hunched appearance. In many cases however, experiments will be terminated before animals experience these symptoms. We are using models that usually result in a self-limited disease.

- **Tumour growth**: will be induced in some animals and followed for up to several weeks. While tumours can in rare instances be associated with additional clinical symptoms like skin necrosis, hypothermia, weakness
, diarrhoea and body weight loss, we will normally terminate experiments before.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

- **Sub-threshold**: The vast majority of all mice (more than 85%) is not expected to experience any pain, suffering, distress or harm that are more than transient or mild.
- **Mild**: A minority of animals (approx. 3.5%) will suffer mild harm.
- **Moderate**: In a further minority of all animals (approx. 8%), we expect to observe moderate severity, usually associated with experimentally induced inflammation, arthritis or tumours.
- **Severe**: We expect only a small fraction (no more than 1%) of all animals to experience severe symptoms, likely associated with advanced rheumatoid arthritis.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 03 March 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project studies complex interactions between immune cells and their environment in both physiological and pathological conditions like pregnancy, tumours and inflamed joints. We aim for a mechanistic understanding of these interactions, their cause-consequence relationships and long-term effects, such as the impact of early exposure to adverse environments on health and disease later in life. Even refined in vitro or ex vivo studies cannot replicate the complexity of the cell-cell communication underlying these processes, or their long-term consequences in such intricate biological systems. Therefore, the need to study these biological responses using in vivo models remains.

Which non-animal alternatives did you consider for use in this project?

We have considered the use of cell lines, primary cell culture (i.e. ex vivo culture of cells derived from human or animal tissues), more complex co-culture systems, in which for example immune cells and non-immune cells are cultured together, as well as organ explant cultures and organoids (i.e. "miniorgans" grown in culture that contain multiple cell types and may model some organ functionality).

Why were they not suitable?

Neither relatively simple approaches like cell lines and ex vivo culture of primary cells, nor more sophisticated ones like organ explants and organoids can fully replicate the complexity of the cell-cell communication underlying immune functions in pregnancy and embryonic development, tumour growth and chronic inflammatory disease. There are two main reasons for that: One, these interactions are not simply binary e.g. between one specific immune and one epithelial cell type. Second, they occur over an extended period of time, e.g. spanning all of gestation, and have long-term consequences on multiple organ systems. Co-culture systems for example are limited to binary or a restricted number of cell-cell interactions, and even though organoids can be cultured for extended periods and contain multiple organ-specific cell types, these are of epithelial nature in the classical models, and immune cells have to be added exogenously in a co-culture manner. Therefore, these non-animal alternatives are not suitable for addressing our scientific questions. However, they may complement in vivo studies and represent promising reductionist models in which to address e.g. the involvement of a specific inflammatory mediator produced by immune cells at the maternal-foetal interface. Moreover, animal experiments will be complemented with in silico approaches wherever possible. Gene expression analyses will maximally exploit published datasets and publicly available databases of relevant immune cell types, tissues and developmental stages, e.g. the ImmGen consortium or recently published single cell atlases for mouse gastrulation and organogenesis, as well as comparable resources for human data.

A retrospective assessment of replacement will be due by 03 March 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

The number of animals we estimate to be used throughout this project is based on statistical and experimental design considerations as well as project-specific ones:

- Our target is the smallest number per experimental group that still has the capacity to generated statistically powerful data. For the sake of this estimation, this number is based on experience, but we will initially perform a formal sample size analysis and pilot experiments wherever this has not previously been done.

- This project generates and uses genetically modified animals. In many breeding schemes, not all offspring animals can be used in experiments, because they do not carry the right genetic modification. These animals are, however, accounted for in the total estimation.

- This also applies to experiments in which pregnant animals are undergoing experimental procedures. In some cases, our scientific interest is in maternal processes rather than the offspring. However, our estimation does take into account any offspring that is produced.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

- For many of the experiments in this project, we or our collaborators have previously established treatment regimens and in some instances have obtained preliminary data that have guided experimental design. In many other cases, the scientific literature has been consulted, in which similar experimental approaches have been reported. For further refinement, we will be closely working with the local veterinary services team and routinely consult the NC3R’s website.

- An experimental design consideration that is of particular importance for this project is that of the experimental unit. In accordance with its definition as "the smallest division of the experimental material such that any two experimental units can receive different treatments”, the experimental unit in experiments addressing the effects of maternal treatments on the offspring is a given pregnancy or an entire offspring litter, and not individual offspring animals. To reduce the overall use of experimental animals with this aspect in mind, we will therefore use the offspring of a given litter for different readouts wherever possible. Conversely, for a given experimental readout, we aim to use individual mice from different litters.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

- Efficient breeding: In many of our experiments, we will use genetically modified animals. Wherever possible will we follow breeding strategies in which most if not all offspring animals can be used for experimental purposes, thereby reducing production of surplus animals.

- For most experimental procedures, treatment regimens have previously been optimised by ourselves, our collaborators or published in the literature. As a starting point, we will closely adhere to these protocols.

- We will also share tissues from experimental animals wherever possible, either for different projects within the group or with other researchers interested in and authorised for studying immune development.

A retrospective assessment of reduction will be due by 03 March 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project uses in vivo mouse models of embryonic development, pregnancy and adult disease. Specifically, we will study pregnancy in normal and conditions challenged by inflammation, chronic inflammatory disease that resembles rheumatoid arthritis in humans as well as tumour development. Although clinical symptoms cannot be avoided altogether, the precise models and methods we will use are designed to minimise pain, suffering, distress and lasting harm to the treated animals.

- **Substance delivery, prenatal inflammation**: For the vast majority of substances that we will deliver, treatment regimens (i.e. dosage, timing and duration, delivery route) have previously been optimised by ourselves and our collaborators, or published in the scientific literature. Where there are several options available we will always opt for the less invasive or more refined method. For example, where substances shall be administered orally and in a non-punctual manner, we will do so e.g. via the food or drinking water. Where substances shall be delivered directly to embryos during pregnancy, we will opt for an ultrasound-guided rather than the classical surgical technique. Treatment regimens will also be tailored to the specific scientific question. For example, we will elicit inflammation during pregnancy, either to study the involvement of foetal and maternal immune cells in preventing preterm birth, which requires stronger inflammation, or in order to study its long-term consequences on offspring immune development and disease susceptibility, for which lower-grade inflammation will be induced. Finally, wherever possible, substances will be provided in sterile solutions.

- **Immune development**: We and others have previously established refined models that allow pinpointing the developmental origins and kinetics of immune cells across the lifespan. For example, instead of using whole-body irradiation, which is known to induce systemic inflammation and often tissue damage or even radiation sickness, we will use refined models in which cells are transferred into unconditioned mice or animals that have undergone only partial irradiation, leaving the rest of the body protected.

- **Arthritis models**: We will use several well-established arthritis models in pregnant and non-pregnant adult mice. These models have distinct advantages for our questions. For example, arthritis induced by administration of serum from already arthritic mice or antibodies generally causes rapid, self-sustained disease are ideally suited to model maternal arthritis with minimal suffering for the pregnant animals. Arthritis induced by immunisation with collagen, on the other hand, represents a valuable model to test if animals that have prenatally been exposed to adversity are more likely to develop arthritis, since the mice we use are normally rather resistant to this model. In all instances we will minimise suffering by environmental enrichment e.g. with soft bedding, which maximises comfort for arthritic animals.

- **Tumour development**: We will use a model in which tumour cells are engrafted into unconditioned, immunocompetent mice. This significantly reduces suffering associated with many other tumour models. The humane endpoints for this model are well defined and take into consideration tumour growth and other clinical symptoms such as substantial weight loss and general weakness, however, these are rarely observed in this particular model.

Why can’t you use animals that are less sentient?

Our aim is to establish a mechanistic understanding of how immune cells contribute to embryonic development, pregnancy, arthritis and tumour development and establish cause-consequence relationships between e.g. deleting a particular cell type and an observed biological effect. We therefore depend on genetic tools, for which mice remain the species of choice. Moreover, stages of immune development are relatively conserved between mammals, and much of our knowledge has been established in mice. They thus represent the best model organism for our scientific questions.

Owing to our interest in immune development, we will study animals at an immature life stage (e.g. embryos or newborns) in many of our experiments. However, we also study long-term consequences of experimental treatments, such as the effects of prenatal exposure to adversity on offspring immunity or disease susceptibility. In these cases, we therefore cannot terminate the experiment at such early stages. Moreover, where we study rheumatoid disease or tumour development, we are often required to monitor disease longitudinally. However, we will always use the earliest possible experimental time point that produces meaningful scientific data, and have implemented well-defined humane endpoints as well as refinements.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The following measures will be applied throughout the project:

- **Monitoring:** In general, all animals will be regularly observed by trained staff and animal caretakers. Experimental animals will be additionally monitored at intervals and by means tailored to the experimental treatment. Immediately following treatment, e.g. substance administration by injection, animals will be inspected for any signs of distress. Animals that have been subjected to disease models will be monitored and clinically scored e.g. every 1-2 days for arthritic and tumour bearing animals. Wherever possible will we use non-invasive means of monitoring that also enable longitudinal analyses. For example, camera monitoring and ultrasound imaging will be used to monitor preterm birth or other pregnancy complications. Ultrasound or e.g. bioluminescent imaging approaches may also be used to follow tumour growth.

- **Surgery and post-operative care**: Although post-surgical infections and death resulting from anaesthesia or surgical complications are very uncommon, in the few cases where surgery is performed, great attention will be paid to post-operative care: Anaesthetics will be used at correct doses in accordance with local and HO guidelines, and body temperature will be maintained post surgery e.g. via heat pads. Pain will be controlled during surgery by general anaesthesia and post surgery by pain killers, unless these interfere with the scientific purpose. Risk of post-surgical infection will be minimised by good surgical and aseptic techniques. Surgical sites will be monitored for signs of inflammation and infection. Post-surgical infection is unlikely and antibiotic cover may be given under the advice of local veterinary services.

- **Pain management**: In both the arthritis and tumour models, mice will be provided pain relief at defined clinical scores, unless these interfere with the scientific question, e.g. where the inflammatory response shall be studied, but is impaired by administration of non-steroidal anti-inflammatory drugs.

- **Animal handling, environment:** Mice will be handled gently and via tunnel handling at all times, and we will also provide an environment enriched e.g. with nesting material. Tumour-bearing and arthritic as well as pregnant animals will be handled with particular care and may be provided additionally e.g. with soft bedding to maximise comfort. Where experimental regimens require repeated treatments, animals may be trained to the procedures prior to initiating the experiment to minimise stress.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

In addition to the aforementioned resources (webpages of local veterinary services, NC3R), which include ARRIVE and PREPARE guidelines, we will specifically follow published guidelines for the welfare and use of animals in cancer research
(*Workman et al. British Journal of Cancer 2010, DOI: 10.1038/sj.bjc.6605642*), as well as refinement in arthritis research (*Hawkins et al. Inflammopharmacol 2015, DOI 10.1007/s10787-015-0241-4*), as well as their updates and similar guidelines published in the meantime.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly consult with local veterinary services and stay informed via the NC3R website as well as other resources concerned with animal welfare in biomedical research). We will also regularly participate in HO "road show" events and those organised by our local AWERB committee.

A retrospective assessment of refinement will be due by 03 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

40.The role of adiponectin in equine endocrinopathic laminitis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

laminitis, adipose-tissue derived hormones, insulin, endocrinopathy

Animal types	Life stages
Ponies	adult
Horses	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To further elucidate the role of specific adipose tissue derived hormones in the pathogenesis of equine endocrinopathic laminitis.
A retrospective assessment of these aims will be due by 16 June 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Laminitis is a painful condition of the equine foot that affects approximately 4% of the horse and pony population in the UK and worldwide. In addition, it is frequently recurrent, with up to 70% of animals suffering from repeated episodes. There are three forms of laminitis, namely sepsis-associated, endocrinopathic and supporting limb laminitis. Endocrinopathic laminitis is the commonest form, accounting for up to 90% of cases and encompasses laminitis associated with the endocrine (hormone) diseases equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID). The key feature of EMS is insulin dysregulation (ID), which is abnormal insulin metabolism in response to a normal physiologic process, such as eating. In horses, this manifests as high blood insulin concentrations (hyperinsulinaemia) and/or an excessive insulin response to ingested carbohydrate and/or resistance to insulin at the level of the tissues. Additional features include obesity, high blood fat concentrations (hypertriglyceridaemia) and abnormal fat tissue metabolism (adipose dysregulation) manifesting as abnormal plasma adipokine (hormones produced by fat tissue) concentrations. It is well established that high blood insulin concentrations for a prolonged period (48-72 hours) can induce laminitis, but the underlying mechanism remains unclear. Current research has focused on insulin binding to and inappropriately stimulating the receptors for the hormone insulin-like growth factor-1 (IGF-1) which are found in the equine foot. Some adipokines have anti-inflammatory and insulin-sensitising actions and low circulating concentrations of some adipokines, as well as high blood insulin concentrations, is a risk factor for endocrinopathic laminitis. In other species, specific adipokine and insulin signaling pathways within cells converge at the level of the adaptor protein APPL1 and there is emerging evidence of cross talk between specific adipokines via its receptors and both the insulin and IGF-1 receptors, resulting in increased and decreased signaling respectively. This project will firstly investigate the effect of high blood insulin concentrations, induction of tissue insulin resistance (using corticosteroids) and obesity (via pasture-induced weight gain) on circulating concentrations of specific adipokines *in vivo*. Human metabolic syndrome (HMS) is very similar to equine metabolic syndrome in terms of the metabolic alternations that occur and it is associated with an increase risk of certain cardiovascular diseases. Dietary manipulation and pharmacologic agents are used to increase circulating concentrations of certain adipokines in people with HMS and this in turn reduces the associated cardiovascular disease risk. Thus, this project will also determine whether similar approaches can be used in EMS. The effect of weight loss with or without dietary supplementation and/or pharmacologic agents on circulating concentrations of specific adipokines will be evaluated. Potential pharmacologic agents will first be screened *in vitro* through evaluation of their effects on equine fat tissue (adipocyte) adipokine production.
What outputs do you think you will see at the end of this project?

This project seeks to further elucidate the role played by specific adipokines in endocrinopathic laminitis and to

identify potential pharmacologic agents and management interventions that will increase circulating concentrations of these adipokines. This in turn may reduce the risk of endocrinopathic laminitis in high risk animals. This new information will be disseminated in the form of presentations at suitable equine veterinary and research conferences and publications in suitable journals.

Who or what will benefit from these outputs, and how?

The project has a potential beneficial welfare impact for horses and ponies worldwide and an economic impact for their owners through reduced veterinary expenditure and athletic performance loss. This impact will not be fully realised until the project is completed.

How will you look to maximise the outputs of this work?

New knowledge gained will be disseminated through presentation at suitable conferences to researchers working in this field, veterinarians and horse caregivers and publication in suitable journals and lay articles. This will include publication of unsuccessful approaches.

Species and numbers of animals expected to be used

- Ponies: 25
- Horses: 25

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

It is not possible to achieve the objectives of this project without using animals, as we are studying complex metabolic pathways and physiological responses, that cannot with our current state of knowledge, be modeled using isolated tissues, cells or computer simulations.
Laminitis is a disease which affects adult horses and ponies and it is therefore most appropriate to undertake these studies in these animals. Whilst we can model some aspects of digital vascular physiology and fat, muscle and caecal function *in vitro, t*he unique metabolism of the horse is central to the pathophysiology of the endocrinopathic laminitis. Thus, our studies to further elucidate the role of specific adipokines in the pathogenesis of the disease require *in vivo* experiments.

Typically, what will be done to an animal used in your project?

Typically, animals will undergo each of the procedures a maximum of twice in this project.
Protocol 1 involves placement of intravenous catheters in each jugular vein under local anaesthesia and infusion of glucose and infusion via one catheter and collection of blood samples via the second catheter. It also involves a single intramuscular injection of corticosteroid followed by blood sample collection via jugular venepuncture.
Protocol 2 involves consuming sufficient pasture to promote natural weight gain. Blood samples will be obtained weekly by jugular venepuncture until each animal becomes overweight. This will be followed by weight loss achieved through consuming a hay-based diet with or without supplementation using nutritional supplements or pharmacologic agents administered orally. Blood samples will be obtained weekly by jugular venepuncture until each animal reaches ideal weight.

What are the expected impacts and/or adverse effects for the animals during your project?

Studies conducted under this licence should not induce long term adverse effects in the animals.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All protocols will be mild for all animals.

What will happen to animals at the end of this project?

- Kept alive
- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 16 June 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is not possible to achieve the objectives of this project without using animals as we are studying complex metabolic pathways and physiological responses, including some that are influenced by season, that cannot with our current state of knowledge, be modelled using isolated tissues, cells or computer simulations.

Which non-animal alternatives did you consider for use in this project?

Isolated equine tissues or cells and computer simulations were considered for use in this project. *In vitro* studies will inform which drugs to use in protocol 3 and help understanding of the mechanisms involved in adipokine release from cultured adipocytes. The combination of *in vitro* and *in vivo* approaches is more powerful scientifically than either on its own.

Why were they not suitable?

Whilst *in vitro* studies will be used to inform the choice of pharmacologic agents used in protocol 3, they are not suitable as they do not take into account the complex physiological responses and complex metabolic pathways that all interact in the pathogenesis of endocrinopathic laminitis.

A retrospective assessment of replacement will be due by 16 June 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals to be included in this project has been based on sample size calculations and on the assumption that animals will be re-used between protocols in order to reduce the overall number of animals used. Data from our previous studies involving measurement of circulating adipokine concentrations in healthy ponies as well as weight gain and weight loss studies have been used to inform these studies. In each case, group sizes of six animals are sufficient. In addition, in previous *in vivo* equine studies, we have found that group sizes of six animals have been sufficient to produce robust results. Protocol 3 requires three groups of animals equating to a total of 18 animals. Thus, it is estimated that 25 animals will be used in total to allow for illness, accidental injury and the need to repeat a study in an individual animal for experimental reasons.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The number of animals to be included in this project has been based on sample size calculations and on the assumption that animals will be re-used between protocols in order to reduce the overall number of animals used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The numbers to be used in this project are based on sample size calculations and previous experience.
A retrospective assessment of reduction will be due by 16 June 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and

methods cause the least pain, suffering, distress, or lasting harm to the animals.

Protocol 1

The methods used to induce hyperinsulinaemia (high blood insulin concentrations) and tissue insulin resistance have been previously validated for ponies. The duration of the hyperinsulinaemia chosen is much shorter than that which has been previously reported to induce laminitis in healthy ponies. In addition, there is no scientific evidence to link corticosteroid administration with the development of laminitis in healthy animals, only in those with other laminitis risk factors. thus, only healthy, ideal weight animals with no history of previous laminitis will be used. Jugular catheters will be placed using local anaesthetic in order to minimise any pain and distress potentially associated with repeated blood sampling.

Protocol 2 and 3

The pasture-induced weight gain will be undertaken in consultation with an equine nutrition specialist to ensure that there is a gradual gain in weight over 9-12 weeks. Previous studies using this approach have not resulted in any adverse effects. The weight loss will be induced by feeding a diet low in non structural carbohydrate (<10% dry matter) at 1.25% (dry matter intake; DMI) of body weight. This is standard dietary change that is recommended by veterinary surgeons for weight loss in clinical cases of equine obesity when owners have allowed their animals to become obese. Thus, this will mimic somethings that happens in the real world. In those animals with weight loss resistance (animals that fail to lose weight despite dietary restriction), the diet may need to be reduced to 1% (DMI) of body weight, but no lower as lower percentages are associated with gastrointestinal disturbances such as gastric ulcers and the development of stereotypies (repetitive abnormal behaviours). The methods that will be used to assess the effect of the weight gain and subsequent weight loss on insulin metabolism (i.e. oral sugar test and insulin tolerance test) are methods that are commonly used in equine clinical practice.
Thus, all of these methods proposed have been previously validated for use in ponies in either the clinical or the research setting. Animals will be returned to their normal management regime between protocols which will involved continuous access to pasture. All of the protocols are mild in severity and none of the methods will cause lasting harm to the animals.

Why can’t you use animals that are less sentient?

As previously explained, all of the experiments proposed need to be performed in horses and ponies. As equine metabolism differs significantly between the foetus, neonate and adult, and laminitis is a disease that only affects adult horses and ponies, we are unable to use a less sentient stage of life.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals will be habituated over several days to the room and the stocks in which the insulin infusion will be undertaken prior to commencement of the protocol.
All animals will be constantly monitored for the duration of all protocols and for the next 3 days to ensure that no adverse effects develop. If any adverse effects are noted, appropriate treatment will be provided immediately in consultation with the named veterinary surgeon.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the NC3Rs advice on the 3Rs for project licence holders.
The NC3Rs General Principles for blood sampling applicable to horses will be followed as well as the recommendations relating to use of vascular catheters.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed of advances in 3Rs via the 3Rs Liaison, as well as via the NC3Rs website and attending relevant seminars/talks. Wherever we find an opportunity to improve our technique/experimental design to minimise animal numbers and/or suffering, we will rapidly incorporate it into our protocols. We will closely work to ensure that our animal care is always optimal, which ultimately ensure high quality results.

A retrospective assessment of refinement will be due by 16 June 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

41. Direct MR and Optical Imaging of neuronal cell swelling.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The project aims to determine if an MRI scanner can be used to directly measure brain activity (via neuronal cell swelling). Currently this is not possible.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Current clinical based MRI methods used to investigate how your brain works do not directly probe brain function. Instead brain activity is inferred from changes in oxygen level in your blood (with cells using more oxygen when active). Unfortunately, to date scientists are unable to fully describe the relationship between brain activity and blood changes. This complicates the use of MRI for studying brain function.

If MRI methods were able to measure brain activity directly it would help doctors learn how normal, diseased or injured brains function. In turn this would help them to track and treat conditions like Alzheimer's or Autism.

A new MRI method, believed to measure changes in brain cell size has been proposed to meet this need. When brain cells are active they get bigger. Our research will use high resolution optical imaging in the rat model to look directly at cell size while we also make MRI measurements. This will therefore confirm or disprove if this new MRI method is measuring brain activity directly.

What outputs do you think you will see at the end of this project?

This project will produce data and information as to whether a new MRI method can in fact measure brain activity directly. The data will be presented to leading brain scientists and doctors via journal articles and at national/international conferences.

Who or what will benefit from these outputs, and how?

Short-term (0-5 year) benefits: The methods developed as part of this project will offer brain scientists a new way, based on brain cell size, to investigate brain function. This presents as a new direction/different strategy for measuring brain function and could offer novel insight into the workings of the human brain.

Medium-term (5 year) benefits: Based on this validated method, new treatments for brain conditions could be proposed.

Long-term (6 year+) benefits: The new MRI method could become the clinical method of choice for looking at brain function. It would allow doctors to map the working brain and detect the effects of tumours, stroke, head and brain injury, or diseases such as Alzheimer's. In addition, it could be used to assess the risk of brain surgery or similar invasive treatment for a patient.

How will you look to maximise the outputs of this work?

Data will be published even if unsuccessful to prevent repetition. We are working closely with a neurosurgeon at a leading hospital NHS trust. This collaborator is keen to see swift uptake of this imaging technology in theatre and explore applications within his patient cohorts. **Species and numbers of animals expected to be used**

- Rats: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The rat brain is a well-defined model for imaging research. Areas with specific functions (responding to for example paw or whisker sensing) are easily identifiable in rats.

Typically, what will be done to an animal used in your project?

All animals will be put to sleep with deep anaesthesia administered via injection. In addition, we will use pain killers to ensure the animals feel no pain. Throughout our experiments the animal vitals will be carefully monitored and maintained.
The animal will undergo delicate surgery. Their skull will be exposed and thinned to translucency so we can see their brain directly. We can then assess with light if their brain cells change size when we touch their whiskers (one of their primary sensing organs – like our hands). MRI scans will be recorded of the animals at the same time and the data compared to light measurements.
The surgical procedures are expected to take around 2 hours. The animal will be in the MRI scanner for no more than 6 hours. Due to the nature of the surgery, once the experiment is complete animals will be killed. However, their brain tissue will be preserved for continued study.

What are the expected impacts and/or adverse effects for the animals during your project?

There will be very little adverse effects of our procedures.
We will use pain killers to ensure the animals feel no pain.
All experiments use non-recovery anaesthesia.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Non-recovery - all animals.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The brain is one of the most complex organs in the living body. No computer can yet model the brains complex biology or function. MRI is non-invasive and safe for human use, but in order to determine whether the new MRI method is indeed measuring changes in brain cell size we must use high resolution optical imaging. Visible light does not pass through the scalp and skull and these must be removed in order for the light to reach the brain. The use of animals is therefore unavoidable.

Which non-animal alternatives did you consider for use in this project?

Imaging techniques have been used on ex-vivo brain slices already.

Why were they not suitable?

Brain slices do not contain live blood vessels. Therefore this model is not representative of what we would image on a person in and MRI scanner.
Operations where human brain is exposed for light measurements are rare and in most cases already demanding procedures. Adding time to make these measurements during surgery is not always ethical.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers were estimated based on previous experience and published data.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The light and MRI measurements can be taken at the same time. This inherently reduces animal numbers (compared to completing separate experiments with each technique).
In addition, by using a long lasting anaesthetic and maintaining animal vitals we can maximise the amount of data obtained from individual subjects, this reduces the overall number of animals needed to meet the project

objectives.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

I have extensive experience in the field of brain imaging. The blood responses we are measuring are typically large and reliable for the given methods. Therefore, if a standard blood response is not measured in response to the stimulus we will know immediately that something is wrong with our setup and the NVS can be consulted immediately.
Following the experiments animals will be killed and brain tissue preserved for further study.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The project uses a non-recovery rat model. Brain function data is collected with MRI and optical/light imaging scanners.
Pain killers and analgesia will be applied in conjunction with anaesthetics to make sure the animals feel no pain which will limit suffering, distress and lasting harm.
If any procedural complications do arise veterinary advice will be sought immediately.

Why can’t you use animals that are less sentient?

Data needs to be collected on the developed/mature brain meaning we need adult subjects.
The physical limitations of the optics and MRI system mean that less sentient species (usually smaller e.g. mouse) would be difficult to work with in terms of both required surgery and size of the equipment involved.
Animals are used at a terminal, non-recovery anaesthesia model.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Pain killers/analgesia will be applied in conjunction with anaesthetics to make sure the animals feel no pain. Top-up doses of anaesthetic will be delivered as required. This ensures animals do not wake up during experiments. All experiments have to be approved by a NACWO and NVS.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

PREPARE guidelines for experimental planning: S*mith, AJ, Clutton, RE, Lilley, E, Hansen KEAa, Brattelid, T. (2018): PREPARE: Guidelines for planning animal research and testing. Laboratory Animals, 52(2): 135-141.*

The Experimental Design Assistant (EDA): https://www.nc3rs.org.uk/experimental-design-assistant-eda
LASA for aseptic practice: http://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The Biological Services Facility at our institute holds quarterly meetings for updates and support for the 3R's. We will also engage with the national centre for the replacement, refinement and reduction of animals in research via their dedicated website, blogs and social media accounts.

Home Office

NON-TECHNICAL SUMMARY

42. Discovery of novel compounds for the treatment of dementia.

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Dementia, Neurodegeneration, Drug discovery, Alzheimer's disease, Parkinson's disease

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our aim is to identify and validate new mechanisms for treating dementia, to identify novel, drug-like compounds that work through these mechanisms and to demonstrate their efficacy in animal models of brain disorders such as Alzheimer's and Parkinson's disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Dementia is a significant global health problem. There are already approximately 50 million people worldwide with dementia, and as the general population ages, this has been predicted to increase to 150 million by 2050. Dementia causes an increasing burden to sufferers, their carers and to society as a whole through the cost of healthcare.

There are currently no treatments available that stop or reverse the progression of dementia. Our project aims to identify chemical compounds that have the potential to be developed as drugs for treatment of this disorder.

What outputs do you think you will see at the end of this project?

Outputs expected at the end of this project

Information on potential targets for drug discovery.

Our first objective is to identify novel "drug targets". These are the molecules in the body which drugs interact with to exert their effects. We need to validate drug targets by demonstrating that interfering with their action causes the appropriate changes in the animal, including reducing the symptoms of disease.

- A successfully validated drug target may be the subject of a drug discovery project for further investigation as part of our internal program.
- Occasionally a drug target may be successfully validated but does not fit within our strategic aims or objectives. In this rare scenario we aim to partner with other organisations or publish the data to generate interest in the wider scientific community.
- Validation of a particular target may be unsuccessful but our data may indicate that the target molecule forms part of a pathway which may include other possible drug targets.
- Where target validation is unsuccessful, this information will also be made publicly available through publication, particularly where there is broader interest in the biology, for example in other disease areas.

Demonstration of compound efficacy in disease models

The second objective is to discover drug-like molecules that may interact with the drug target and to show that these molecules reduce disease symptoms in animal models of dementia.

- If a drug discovery project results in a novel drug-like compound with efficacy in a relevant model for dementia this will initiate efforts to further develop the compound towards clinical testing in humans, for example through partnership with a pharmaceutical company.
- Compounds that do not achieve efficacy, but nonetheless have appropriate drug-like properties may be repurposed for use in other disease areas or as tool compounds for research.

Who or what will benefit from these outputs, and how?

Data on new drug targets may be available in the short term and will benefit our Institute through feeding into more long-term objectives or may benefit the wider field on neurodegenerative disease. We collaborate with other groups working in the same field and may share data, samples, reagents and animal models with these groups. (1-5 years).

Favourable data on compound efficacy that leads to clinical research will take longer to generate, but may have a major impact on drug discovery for dementia, due to the current lack of research in this area within the pharmaceutical industry. (3-10 years).
It is our intention that compounds we develop will eventually reach approval as drugs for dementia. The likelihood of this occurring is relatively low but the benefits to society would be considerable. (10-15 years).

How will you look to maximise the outputs of this work?

Our institute is an academic group with expertise in preclinical drug discovery methodologies used within the pharmaceutical industry and applying these in an academic setting. We work with a wide network of collaborators in the academic sector to investigate and validate interesting and novel drug targets and have strong connections within the pharmaceutical industry to facilitate the eventual development of these ideas into drugs to benefit patients suffering from neurodegenerative disease and dementia.
As an academic group we seek to publish our findings whenever possible.
Chemical structures of molecules originating in our laboratory and the properties of these molecules may be published in the form of patents. This is necessary to protect intellectual property, but also provides a valuable source of information on the viability of drug targets and chemical structures and activity to the wider pharmaceutical industry.
Where projects are unsuccessful or no longer of interest to us, we will seek every opportunity to disseminate the work at scientific and public meetings as well as publish data in scientific journals.
Species and numbers of animals expected to be used

- Mice: 20,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

All of the procedures on this project will be carried out using adult mice. Some of the mice used in our studies will have genetic modifications. In the majority of cases this will not have any impact on the health and wellbeing of the animals. In some cases we will need to use mice that have been genetically altered so that they develop symptoms of neurodegenerative disease.

Typically, what will be done to an animal used in your project?

There are several different scenarios which a mouse may experience, the following are examples:
Example 1: In many cases mice will be given a single dose of a compound by injection. After an interval of up to 48 hours the mouse will be given a terminal overdose of anaesthetic and a blood sample withdrawn from the heart.

Example 2: Genetically altered disease model mice will have their diet reduced at 6 weeks of age and be tested in touchscreen apparatus daily for three weeks. This comprises an automated testing system for learning, memory and other aspects of cognition in which mice are taught to respond by touching an image on a screen in return for a food reward. The mice will then be dosed with a virus to induce a genetic change in the brain. This will be done through a vein in the tail while the mouse is anaesthetised. The mice will be tested again in the touchscreen apparatus once a day for five weeks. At 15 weeks of age they will be given an overdose of anaesthetic and killed by perfusing a substance through the heart to preserve the animal's tissues for microscopic examination.
Example 3: At six weeks of age mice will undergo surgery under general anaesthetic to inject a substance directly into the brain. The mice will be allowed to recover for two weeks, and then will be dosed with a substance once a day using a feeding tube through the mouth. Over the same period, behavioural tests will be carried out once a week to test their ability to remain on a rotating cylinder and to cross a narrow beam. During this time the mice are expected to experience a gradual deterioration in their movement abilities, experiencing tremors and impaired coordination. At the end of the dosing period the mice will be given an overdose of anaesthetic and killed by perfusing a substance through the heart to preserve the animal's tissues for microscopic examination.

What are the expected impacts and/or adverse effects for the animals during your project?

Dosing either by injection or through a feeding tube and blood sampling will cause mild pain and distress to an animal, but this will be short-lived.
The techniques used for behavioural testing in this project are not expected to cause pain or distress other than transient stress due to being handled. For some behavioural tests the animals' food is restricted. This will lead to weight loss, but the general condition of the animals is not expected to be affected in other ways.
Surgical techniques involve a greater degree of pain and distress which may last for a few days after surgery. Pain relief will be given routinely to animals undergoing surgery. Occasionally (less than 2%) a surgical wound may fail to heal properly, in which case the named veterinary surgeon will be consulted. One attempt may be made to re-close the wound if this is within 48 hours of surgery, otherwise the animal will be humanely killed.

Most genetic alterations to animals used in this project do not result in any apparent change to the wellbeing or behaviour of the animals. In some of the disease models that we use, genetic alterations do lead to a progressive impairment in the animal's ability to function normally, especially where these lead to impairments in the animal's movement (loss of balance and coordination, tremors). Typically these symptoms worsen over a number of weeks (depending on the model). Once symptoms appear, mice are monitored at least three times per week and the mice are humanely killed when specified limits are reached.
Dosing of substances to induce disease symptoms may result in similar changes to the genetically altered models mentioned above. We will monitor these animals in the same way, and apply the same limits before humanely killing the animals.
Some of the substances we dose will be completely novel, and may have potential unknown side effects. These substances will be dosed at low doses initially and the animals will be closely observed for any adverse effects before we proceed to higher doses.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severities are mild (approximately 75% of mice) and moderate (approximately 25% of mice).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

When investigating novel drug treatments for disease it is necessary to understand how manipulation of the target for the drug impacts on the biology of the whole animal. There may be unwanted effects of drug treatment in other tissues or organs which would indicate a risk of side effects if the drug were to be dosed in humans. Furthermore, we need to gain a thorough understanding of what happens to the drug when it enters the body, including the rate it is absorbed from the gut into the bloodstream, where it is distributed in the body and how quickly it is eliminated.
When considering the effects of drugs or manipulating drug targets in the brain it is important to recognise the unique complexity of this organ, with many different cell types and networks of connections between nerve cells which cannot be fully replicated outside a living animal. Furthermore, if we want to understand how potential drugs may impact on symptoms of dementia we need to be able to test their effects on learning and memory in mouse models of disease.

Which non-animal alternatives did you consider for use in this project?

We have considered, and already use many non-animal alternatives wherever possible. These include assays using cells grown in culture for testing the activity of potential drugs and understanding the basic biology underlying their mechanism of action. In addition we make extensive use of computer based modelling of drug properties to understand how the structure of drug molecules may affect their interactions within the cell and how they are likely to be absorbed and cleared from the body.

Why were they not suitable?

These approaches are suitable for much of the work that we carry out. However, in order to fully evaluate novel drugs and their targets, which may have the potential to progress to studies in human volunteers, it is necessary to gain a thorough understanding of how they behave in a whole organism.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This number is based on estimates of:

- the number of projects that we anticipate running the expected number of studies to
- validate and test potential drugs for each project

- the number of animals needed per study, using the minimum number of animals to give a

statistically valid result

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In designing experiments there are several factors that we will use to reduce unwanted variability in our data, this will reduce the number of animals needed to show that a result is not just due to chance:

- The number of animals used per treatment group will be carefully worked out using data from previous studies to carry out these calculations
- Randomisation when assigning animals to treatment groups to avoid unwanted bias from factors like cage differences, time of treatment
- When appropriate, use of baseline data when assigning animals to treatment groups to ensure that baseline measurements between groups do not differ
- We have many years of experience in study design and statistics within our group and in addition we will draw on support from colleagues who are specialists in biostatistics.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Other steps we will take to reduce the number of animals used in our project:

- Using animals from the same colonies, or if possible the same litters for comparison, to ensure that genetic variation between animals is kept to a minimum
- Pilot studies will be used to determine the optimal conditions for experiments, e.g. the appropriate dose levels of drugs, the length of treatment time, the age at which experiments should be started in mice
- Where possible the same individual will carry out procedures, collect and analyse the data within a single experiment, and this person will be blind to the treatment which each animal receives
- Wherever possible we will collect multiple samples from each experiment to avoid unnecessary repetition of studies, these include taking samples to determine drug efficacy, measure drug levels within tissues and investigate possible side effects all from the same experiment
- Where possible we will share information, biological samples and mouse strains with other researchers in the field.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Wherever possible, non-genetically altered mice will be used in studies.
When genetically altered mice are used we will take a number of steps to avoid harm to these animals:

- In some cases the genetic alteration can be "switched on" by giving a drug either by injection or in the food. This ensures that any harm resulting from the genetic modification is experienced only during the experiment, rather than for the lifetime of the animal

- Genetically altered mouse models of disease show a progressive worsening of symptoms with age. When using these mice we will use models with the mildest symptoms and at the earliest age that are compatible with our scientific objectives.

- When using animals that have been dosed with a substance to induce disease we will carry out pilot studies to determine the minimum doses and length of time necessary for treatment that are compatible with our scientific aims.

Surgery to dose a substance directly into the brain will only be carried out if the substance cannot be dosed non-surgically by a different route, and all surgery will be carried out under general anaesthetic using aseptic techniques to minimise the chance of infection. Medication will be given for pain relief and to prevent infection.

Why can’t you use animals that are less sentient?

Adult mice will be used in our project. Mice are regarded as a good experimental substitute for humans as much of our genetics, anatomy and physiology are closely replicated in this species. Furthermore, there are existing mouse models of neurodegenerative disease which replicate many of the features of these disorders that are seen in human patients. Adult mice need to be used as the brain does not complete development until adulthood.
We already carry out studies in lower animals (zebrafish) which contribute to this program of work. This is carried out through a collaboration on a separate licence.
It is not possible to carry out our experiments in anaesthetised animals as due to the length of time the studies typically last.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Examples of refinement in our experimental procedures include the following:
rTG4510 mice are a model of a group of diseases known as tauopathies. These miceshow a tendency to be hyperactive, causing them to run inside the cage. This behaviour can occasionally cause injury through the bedding becoming tangled around the leg of the animal. We will use a softer bedding type to prevent injury. We will also investigate providing running wheels for these mice.
In some of our studies, we may need to dose substances using a small pump placed under the skin. We will adopt as standard practice the soaking of these pumps in saline overnight prior to implantation so as to reduce the formation adhesions in which the pumps stick to the skin of the animals. All surgery is completed using aseptic techniques. Local anaesthetics are given after surgery and monitoring in a separate recovery room is

also carried out to ensure animals return to normal activity before being transferred back to normal housing. Following surgery and re-housing, they are monitored for activity, weight and wound healing for up to 7 days. This is recorded on welfare monitoring sheets.
Compounds are also delivered via oral gavage through a flexible tube into the animal’s stomach. The tip of the tube is dipped in a sweet solution before starting the procedure to make it more palatable for the mice to swallow.
In genetically altered mice that do show disease signs, such as a mouse model of Huntington’s disease, welfare is regularly monitored. Since a major symptom of disease progression in these animals is weight loss, we use this to monitor welfare. They are weighed at least weekly, increasing to daily as the disease signs become clear. Before weight loss reaches 15% of maximum body weight animals are humanely killed.
When administering drugs in the animals' diet it has been found that the texture and composition of the modified diet is different from the regular diet which the mice usually eat. This has sometimes led to aversion to the modified diet and reduced food, and drug intake. When dosing drugs in the diet, we will acclimatise the mice to the modified diet without the drug for a number of days before switching to the diet with the drug.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

PREPARE guidelines checklist for designing and planning experiments
ARRIVE guidelines to ensure reporting quality of data is optimised
LASA Guidelines for Aseptic Surgery - https://www.lasa.co.uk/current_publications
NC3Rs - https://www.nc3rs.org.uk/3rs-resources

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Regular awareness of developments via websites of organisations such as NC3Rs, LASA.

Communications from internal supporting services, via the internal 3Rs research tool and through consultation with Named Persons.

43. Drug Abuse-related Tests

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Drug abuse liability, Addiction therapy, Mechanism of action, Behavioural responses

Animal types	Life stages
Rats	adult, juvenile
Mice	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To support the overall assessment of a new medicinal treatment for an abuse potential and/or to assess its potential to treat substance use disorders or reduce the risk of abuse.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Substance use disorder is a growing and global concern. In 2019 (England), there were 7,376 hospital admissions for drug-related mental and behavioural disorders, 9.4% adults (16 to 59) had taken an illicit drug in the last year, 20.3% of young adults (16-24) had taken an illicit drug in the last year, and there were 2,917 deaths related to poisoning by drug misuse (NHS Digital, UK). In the USA, there were approximately 71,000 overdose deaths between 2018 and 2019 (400,000 deaths between 1999 to 2017).
In the UK, alcohol abuse has a significant impact on health, with an estimated 586,780 dependent drinkers in England alone. Alcohol consumption is associated with medical conditions including mouth, throat, stomach, liver and breast cancers, high blood pressure, cirrhosis of the liver and depression. Liver disease has increased by approximately 40% over the last decade and hospital omissions for mental disorders associated with alcohol by 150%; accidents, violence and general risk taking behaviours also contribute to alcohol-related harms.
A survey carried out involving 8518 drug users in Scotland to determine the most popular drugs of choice (individuals could select more than 1) found cannabis at the top of the charts with 78% of drug users stating that they had taken this drug in the last 12 months. This was followed by cocaine or crack (70%), MDMA (46%), prescription medication (29%) and Valium or diazepam (27%) - (source: Addiction Scotland). Such findings are not geographically restricted, with similar trends seen worldwide.

Drug abuse not only has a direct negative impact on the health and well-being of the individual, it has an adverse effect on industry, education, family, and child development. It also is associated with violence, crime, financial problems, housing problems, homelessness, and the spread of transmissible disease such at HIV. The latter largely a result from the sharing of needles and/or promiscuous risk taking behaviours whilst under the influence of drugs.

For many people, the gateway into substance abuse starts with prescription drugs, such as analgesic, anti-depressant, anxiolytic and attention-deficit/hyperactivity disorder drugs. The on-going opioid crisis has largely resulted from the excessive prescribing of opioid analgesics.
On occasion it may be necessary to reassess a particular marketed medicine for an abuse liability. An example of this is Gabapentin, a treatment for neuropathic pain and epilepsy which was first approved as an uncontrolled drug by the FDA in 1993. However, in recent years Gabapentin (street name 'Gabbies') has become increasingly misused and abused for its euphoric effects and as a result, in 2019, Gabapentin was reclassified as a Schedule 3 controlled drug.
In order to fully understand the risk to human health from an abuse or misuse viewpoint, all new CNS active drugs (including approved drugs looking to be approved in different counties (for which an abuse liability assessment according to current regulatory guidance has not previously been assessed), or those with a change of formulation, indication or route of administration) require to be assessed for an abuse liability risk. This information is important for practitioners to know so that prescribing can be carefully controlled, and patients can be withdrawn off medications in such a way that a withdrawal syndrome is avoided.
With the increasing addiction rate across a broad range of drug classes, treatments for substance use disorders are required. These include the development of drugs with an abuse deterrent formulation; drugs with equal effectiveness, but lower abuse potential; drugs to prevent relapse; and those intended to counteract overdose.

What outputs do you think you will see at the end of this project?

Reports and data which will be submitted for support the regulatory filing of new drugs.
The refinement of models and associated publications sharing such data and best practices.

Who or what will benefit from these outputs, and how?

The primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to informed decisions, based upon data generated in these studies, regarding the risks and/or benefits when humans are exposed to medicinal products.
Achievement of the objectives of this Licence will enable medicinal products to progress into clinical testing and onwards to marketing authorisation and ultimately improving the health and welfare of humans. Without these pre-clinical studies, progression of new medicines to early human studies and on to patients/marketing could not occur. There is also a major benefit when using early drug selection type experiments, as the most promising compounds for further development will be selected and then fully tested so that they can reach the market sooner and as such benefit the health and welfare of humans. The work carried out under this project licence will 1) reduce the risk of new medicines entering the market for which there is an unknown associated abuse liability, 2) help identify potential treatments for substance use disorders, and 3) identify medicines (e.g., analgesics) which have a lower abuse liability risk than many presently used to treat pain.
The use of the experiments described in this licence may indicate major safety concerns or lack of effectiveness with the substance under evaluation at an early stage thus precluding requirement for additional experiments after these screening studies. This can greatly reduce the number of animals required in a programme of work.
In addition, scientific knowledge gained in one programme of work will often be applied to future experiments in order to reduce animal numbers and/or reduce pain and stress to those animals used in the subsequent work, or to target investigations to a particular organ or tissue during toxicity testing or clinical trials.
Sponsors will benefit from work undertaken within this project licence, by obtaining data which allows them to make decisions on the development of the drugs and to support regulatory filings.

How will you look to maximise the outputs of this work?

Where confidentiality permits, data, study design and best practice will be openly shared at conferences, workshops, webinars, blogs and publications.

Species and numbers of animals expected to be used

- Mice: 7000
- Rats: 12000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Animals used during the course of this licence will be rodents. There is wide knowledge of the behavioural response of rats and mice in response to a wide range of substances and a wealth of background literature.
Rats are large enough to provide repeated blood samples for toxicokinetics, thus requiring significantly fewer rats than mice to achieve
the same objective. Mice may be used when considered a more appropriate species, this may include availability of test material which may be limited during the early phases of drug development. Mice may also be appropriate if previous work has been carried out in that species.
In the majority of cases, rodents are the preferred and regulatory acceptable species for the work carried out under this project licence.
Adult animals will be used.

Typically, what will be done to an animal used in your project?

Studies conducted under this project licence fall into the following main categories in terms of procedures and observations. In all cases, upon completion of testing the animals are humanely killed. **1) Learned behaviour in response to positive reinforcement (reward).**

Animals used under these protocols may be surgically prepared with an indwelling cannula to allow for the intravenous administration of the reward. Animals are placed in operant chambers where they are able to access and press levers, which when selected correctly provide a reward (e.g. cocaine or sucrose). Blood samples may be taken on occasion so that test agent exposure levels can be determined and related to the human situation. These tests are used to

- Determine if a new medicine has a rewarding effect and as such a potential abuse liability. Assess the potential of a new drug to modify drug taking (addiction therapy).
- Determine if a new medicine produces effects, as perceived by the animals, as being similar to those of a known drug of abuse.
- Due to the lengthy training associated with these tests, the duration of these studies is many months (e.g. 4- 12 months). On rare occasions animals used for this tests may be re-used on one occasion, providing that they are not suffering from adverse effects. Re-use avoids the need to use naive animals which would need to undergo the extensive training procedures and surgery.

2) Tolerance, dependence, withdrawal

The majority of animals within these protocols will be administered test agents as a daily administration for up to 4 weeks. At various time-points animals will be observed for physiological or behavioural changes (e.g. body temperature, body weight, reaction to a stimulus, and general activity). Blood samples may be taken on occasion so that test agent exposure levels can be determined and related to the human situation. These tests are generally used to

- Determine if a new medicine has the potential to induce a withdrawal syndrome ('bad feelings') upon cessation of dosing.
- Determine if the pharmacological effect (e.g. analgesia) of a new medicine reduces with duration of dosing
- Determine if a new medicine can prevent the development of a withdrawal syndrome.

3) Analgesia

The majority of animals within this protocol will be administered test agents on a single occasion, with a study duration of less than 1 week. The response of the animal to either a thermal or mechanical (pressure) stimuli will be assessed as an indication of analgesic efficacy. On occasion, repeat dosing may be employed in order to assess the development of tolerance and withdrawal, along side efficacy and by doing so, reduce the need for a second study to be conducted to address these potential side effects.

4) Cannabinoid

This test is conducted to assess the potential of a new medicine to produce cannabinoid-like effects (CB1 activity). Animals are dosed on a single occasion. At a single, defined time-point post-dose, the animals are subjected to a thermal or mechanical (pressure) stimuli in order to assess analgesic effects, body temperature, locomotor activity and their reluctance to immediately move when placed in a 'sitting' position. The typical duration of this study type is 1 day.

5) Behavioral sensitisation

Animals used for this purpose are typically dosed (sensitised) on 6 occasions, followed by a week for example of no treatment before receiving a final challenge dose. At defined time-points, locomotor activity is recorded, typically on each day of dosing, for 2 hours on each occasion.

This test is conducted to assess the potential of a new medicine to induce behavioural sensitisation as demonstrated by an increasing trend of hyperactivity with each dose, or to see if it prevents the sensitisation effects of a known drug of abuse. Data may be used to determine if the new medicine has a 'flag' for a potential abuse liability or has the potential to be used to treat addiction, respectively.

What are the expected impacts and/or adverse effects for the animals during your project?

Humane endpoints as documented within this project licence will be applied to animals used under the protocols specified in this licence.
These studies are typically conducted following early toxicology/pharmacokinetic studies and as such the dose levels can be carefully selected in order to avoid undue toxicity. The majority of the protocols within this licence are behaviourally based (e.g. require animals to learn to lever press for a positive reward) and as such adverse effects are not desirable.
For a number of models performed under this licence surgical implantation of catheters is required. In such cases animals are anticipated to show minimal or no adverse effects resulting from surgery following recovery; analgesics will be provided as required throughout the recovery phase.
Animals within this licence may regularly experience the psychoactive effects of known drugs of abuse, and on occasion the associated withdrawal effects once dosing has ceased. The actual effects seen during dosing are largely drug and dose-level dependent. In the case of stimulants at low doses a slight increase in activity may be observed, whereas, at higher doses (rarely used) stereotype behaviours may be seen; withdrawal effects from stimulants are subtle and short lived. For cannabinoids, low doses are not expected to produce overt effects, whereas at higher doses, decreased activity and temperature, analgesia and cataleptic-like behaviour may be seen; withdrawal effects are difficult to detect. Opioids are associated with analgesia, freezing behaviour and straub (elevated) tail; withdrawal effects are pronounced, although relatively short in duration (peaking within 48 hours of dose cessation) with unkempt appearance, decreased activity, and increased sensitivity to touch being key features. For studies conducted under this project licence any such effects are transient, and in general, with repeat dosing begin to lessen.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The severity limit specified (moderate) is considered to be the minimum commensurate with achieving study objectives. For the majority of studies conducted under this licence, the severity limit is moderate due to the cumulative procedures required to achieve the protocol objectives. Repeat dosing, surgical cannulation for dose administration and transient restricted access to water during test sessions (e.g. whilst in respiratory chambers, photobeam activity cages, or operant chambers), are primarily responsible for the moderate severity imposed. In the majority of cases animals will not experience more than mild signs as a result of administration of the test agents.
Marked effects anticipated are primarily those related to the known pharmacology of drugs e.g. sedation or stimulation.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

As a service provider we do not own the test materials under evaluation, therefore, in silico screening tools for candidate selection are not appropriate, however, available literature is searched prior to commencing any in vivo procedures. Prior to conducting in vivo studies, sponsors are requested to provide information relating to their test material candidate, together with details of other work performed, relevant regulatory requirements and a justification for conducting in vivo investigations.
Due to the studies within this project requiring the assessment of behavioural and learned responses, the use of animals is essential.

Which non-animal alternatives did you consider for use in this project?

Regulatory guidance documents require non-animal studies to support the data obtained from the animal studies within this licence. These include in receptor binding/function assays, transporter and ion channel effects and chemical structural evaluation.

Literature searches were conducted to determine if any non-animal alternatives were available, however, as these studies rely heavily on behavioural responses, such studies are not presently available. Where possible, data from in vitro assays are used to optimise the design of the animal studies.

Why were they not suitable?

Non-animal studies alone are not sufficient to fully evaluate the potential abuse risk of a drug or its effectiveness as a treatment for substance abuse disorders.
To fully assess the pharmacodynamic effects (effects of a drug on the body) of a new drug testing in animals is necessary. Only in a fully operational circulating system can the drug's distribution, metabolism, excretion which may alter or intensify the efficacy or adverse effects of the new medicine be fully understood. In addition, behavioural effects can only be assessed in live animals.
For these reasons animal models remain essential in the development and safety assessment of new medicines.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The total estimated numbers of animals to be used have been determined based on previous projects over the past 10 years, regulatory trends and anticipated future requirement, and in review of future services being developed and offered globally for regulatory non-clinical studies.

What steps did you take during the experimental design phase to reduce the number of animals being

used in this project?

Studies will be designed under this licence such that the minimum number of animals will be used in order to obtain the maximum information, whilst the scientific objectives of each study are met, in accordance with regulatory requirements and agreed standard practices.
Data generated from pivotal studies will be statistically analysed, with comparisons drawn between control and treated groups, and reported in a format consistent with the ARRIVE guidelines published by the NC3Rs.
For studies within this project which are primarily designed for efficacy or investigative purposes, side effects may be detected which if considered unacceptable for the therapeutic area for which the drug is intended may lead to the cessation of its development early on. Having the opportunity to terminate the development of a drug as early as possible in the development process reduces the unnecessary use of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Our Company has a wealth of experience and knowledge regarding regulatory requirements and the industry standards used, hence we are in an ideal situation to influence study designs when discussing requirements with our sponsors and will always consider ways of reducing animal requirements. In-house access to professionally trained statisticians is available who are able to help with study design.
Study protocols are reviewed by the AWERB against known guidelines and the Company's ethical compliance policies. For regulatory studies, guidelines require the appropriate number of groups to clearly demonstrate the presence or absence of effects of the substance; core study designs are based on international guidelines where these exist. If not, use is made of literature and scientific principles of experimental design. Where appropriate, use is made of removing or limiting control groups and challenging the need for the use of both sexes. Additional justification will be requested from the study sponsor and reviewed by the PLH/AWERB for studies requiring a greater number of animals in order to better characterise the variability of a scientific endpoint.
Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies, with power-sample size calculations performed for specific studies if necessary to determine group size. Where group sizes allow, data are analysed statistically. However, due to the behavioural nature of studies within this project licence test agent effects are made by examination of data from each animal, rather than or in addition to simply assessing group mean values and statistical parameters.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The animals used during the course of this licence will be rodents. The models within this licence are behaviour based and as such animals are required to be in a good state of health in order to perform tasks associated with some protocols (e.g. lever pressing).
Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.
Individual studies will be designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

Surgical cannulation of animals for the administration of test agents or blood withdrawal has been refined in-house by adopting the pinport method for tether attachment. This method allows the animals to move freely within the home cage, and where possible allows animals to remain socially housed.

Why can't you use animals that are less sentient?

Rodents are the animals with the lowest neurophysiological sensitivity that allows the aims of the studies to be met. Scientific opinion, including that of the regulatory agencies, indicates the use of rodents within these study types are appropriate, and to use other species should only be done if there is strong scientific justification to do so.

The studies within this licence are strongly behaviourally based and as such require the use of conscious animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The severity limits specified are considered to be the minimum commensurate with achieving study objectives. Studies conducted under this licence are within the moderate severity category and it is expected that the majority of animals will have an actual severity that is the low end of moderate or mild, with the actual severity being largely determined by procedural effects (such as surgery or repeat administration) rather than clinical signs resulting from administration of test agents.

All procedures are kept to the minimum commensurate with the study objective. Best practice guidelines for all animal care and use are followed.

In several protocols it is necessary to take serial blood samples from the study animals to monitor plasma levels of the test compound. Where blood samples are required we will take them using the sampling site, volume, and frequency that has least welfare impact on the animals. Where possible samples will be taken under non-recovery anaesthesia. Micro-sampling (using very small volumes of blood, which causes less distress to an animal) will be utilized where methodology is available to do so.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Technical, scientific and regulatory developments will be monitored throughout the duration of this licence. Opportunities to introduce refinements will be evaluated by the Project Licence Holder and Animal Welfare Ethical Review Body to ensure the regulatory and scientific objectives of individual studies can be met whilst achieving the intended benefit. If required, validation studies will be conducted under this licence to ensure these objectives can be met.

Surgical procedures will be conducted in accordance with LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery, LASA, 2017. Analgesia following surgery and other procedures will be provided in accordance with veterinary advice.

Prior to the start of a particular programme or study, the client will be requested to provide information regarding known or potential effects/drug interactions that could be confounded by routine veterinary

treatments or standard husbandry practices. Any such interactions are rare but with pre-planning, alternative treatments may be available (e.g. opioid vs NSAID analgesia, different bedding types etc).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Information issued by regulating authorities, the Home Office, cross company consortiums, in-house projects and scientific associations (e.g. the NC3Rs) will be reviewed and adopted as appropriate in accordance with best practice.
The methods used (dosing, blood sampling techniques, handling and restraint, use of analgesia) will be reviewed during the life time of the licence and any refinements developed will be implemented.

Home Office

NON-TECHNICAL SUMMARY

44. Dynamic regulation of immune responses

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Immunology, antibody responses, lymphocyte migration, pulmonary immunity, splenic immune responses

Animal types	Life stages
Mice	adult, embryo, pregnant, juvenile, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To identify mechanisms that regulate the generation of long-lasting antibody-mediated protection from pathogens.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Effective activation of B cells leads to the generation of highly specific and long-lasting antibody responses. This process is fundamental for host defence and is the **basis for nearly all currently available vaccines**. However, despite their enormous protective potential, antibody responses against several clinically important viruses such as influenza, respiratory syncytial virus (RSV), tuberculosis, HIV and other pathogens fail to confer long-term protection. **This poses a significant medical challenge and highlights the need to better understand the mechanistic basis of humoral memory.**
While antibody responses are critical for protection from primary and secondary infections, abnormal differentiation of pathogenic antibodies can lead to chronic diseases such as **allergy and autoimmune disorders**. Thus, defining the specific pathways that control this process remains an important challenge for the development of therapies against such disorders.
In this proposal, we aim to provide deep mechanistic insights into key steps that lead to induction of long-lasting antibody responses within secondary lymphoid tissues (e.g. lymph nodes and spleen) and to identify factors that regulate the delivery, maintenance and timely termination of these responses in peripheral sites (e.g. the lung). Our main focus is to understand how cell trafficking and interactions with defined niches within living tissues regulate these events, leading to a balanced and efficient immune response.
These studies will have important implications for our ability to **develop novel vaccines that will induce better antibodies with long-term immunological memory against pathogenic microbes.** In addition, these studies may also promote our understanding of how abnormal antibody response develop and persist, **knowledge that may help to identify new strategies to treat immunological disorders (e.g. autoimmune diseases, allergy, chronic inflammation).**

What outputs do you think you will see at the end of this project?

The main goal of this PPL is to enhance our understanding of mechanisms that regulate the generation and maintenance of antibodies. We expect this output to be disseminated via publications in peer reviewed journals and conferences.
Who or what will benefit from these outputs, and how?

In the short run, our studies will advance our understanding of the immune system by addressing key questions that are currently poorly understood:

- How splenic immunity is orchestrated- Long-term immune responses are induced within secondary lymphoid organs, including the lymph nodes and spleen. Among those, the spleen is the largest immunological organ, which contains the greatest number of lymphocytes. As such, targeting immune responses to the spleen may be a powerful approach to induce a strong and effective immune response, for example when vaccinating against challenging pathogens (e.g. influenza virus, tuberculosis, malaria). To induce such a response, it is essential that lymphocytes are able to access specific compartments within the spleen. In this project, we aim to identify the molecules that regulate this process, and which allow immune cells to migrate into the spleen and become activated effectively. Better knowledge of the

basic mechanisms that control these events will be important for future efforts to develop approaches to regulate immune responses for therapeutic purposes.

- How regional humoral immunity is regulated in the lung- Memory B cells play a critical role in our ability to resist repeated infections with the same pathogens and their development is important for the success of most current vaccines. Following infection with influenza virus, a subset of memory B cells has been identified to develop within the infected lung. Localization of memory B cells directly near regions, where viral entry occurs, provides them with a great advantage to respond to new infections quickly and efficiently. However, the molecular mechanisms that allow the cells to remain and survive in the lung are not known. It is also not clear how these cells function during exposure to a secondary infection. In this project we aim to address these questions and to identify molecules that regulate lung memory B cell development, survival and function.

In the longer run, our studies may lead to development of therapeutic agents that aim to enhance or inhibit antibody responses and to prevent cell recirculation (i.e. ability of T and B cells to continuously move from one secondary organ to the next). This may help to:

- Develop ways to treat autoimmune diseases, or to block entry of cancer cells into sanctuaries located within secondary lymphoid tissues.
- Guide the development of vaccines that enhance immune responses in the spleen in order to use its enormous immunological potential.
- Guide the development of vaccines aiming to enhance local antibody production in response to influenza virus and possibly other respiratory pathogens.
- Lead to the development of possible therapeutic agents to antibody-mediated lung allergy.

How will you look to maximise the outputs of this work?

The outputs from this work will be maximised through the dissemination of our findings in publications and conferences. We also aim to expand our collaboration network with the industry (e.g. we are currently in discussion with a US based company aiming to use our recent findings to improve the capacity of CAR T cells to traffic into tumours).
In addition, we will continue to publish our negative results (when relevant) primarily in supplementary data section of manuscript in hope to minimize redundant work.

Species and numbers of animals expected to be used

- Mice: 28000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse is a suitable animal model for our scientific questions because many of the molecular factors that regulate lymphocyte migration and interactions with other cells (e.g. key chemokine receptors, structural elements such as the high endothelial venules, integrins etc) are highly conserved between mice and other vertebrates including humans. This high level of similarity allowed the translation of studies performed in mice to clinical use in humans (e.g. Fingolimod/Gilenya™, which blocks the chemotactic receptor S1PR1. This drug was developed and studied extensively in mice before being approved as the first oral treatment for multiple sclerosis).
The overall structural organisation of lymphoid organs (including lymph nodes and spleen) and the mechanisms that underline B cell activation are very similar in mice and humans. This, again, allows researchers to use mice-based studies as a baseline to generate clinically relevant agents (e.g. vaccines and adjuvants).
Adaptive immunity has evolved in vertebrates and the maturation of the cells and structures that regulate it mature over time (typically 8 weeks in mice). It is therefore not possible to use lower organisms or embryos to address In some cases, procedures may be applied on juvenile or embryos mice in order to induce specific changes early during development. However, the end point of such experiments will be explored in the adult animal.

Typically, what will be done to an animal used in your project?

Many of the animals in this project will be immunized with vaccine-based agents or infected with live pathogens such as Influenza virus, Salmonella or Listeria monocytogenes. In most cases, immunizations are not expected to cause significant discomfort that goes beyond that which is associated with vaccination in humans. Infection with live pathogens is expected to cause disease that will resolve within 7-14 days and which is not associated with mortality. Mice that are infected with virulent Salmonella strains will be killed within 5 days post infection, at a time when the clinical symptoms do not exceed the moderate severity limits.
Some mice may be subjected to re-challenge. Importantly, re-challenged mice are expected to develop a-symptomatic disease due to the existence of immunological memory induced in response to the primary infection or immunisation.
Some mice may be subjected to whole body irradiation and bone marrow transplantation. This procedure is associated with a 14 days recovery period. Symptoms associated with this procedure are very similar to those observed in humans receiving bone marrow transplantation.

What are the expected impacts and/or adverse effects for the animals during your project?

Following infection with live pathogens or bone marrow transplantation, mice are expected to lose body weight and to experience general discomfort leading to symptoms such as temporary reduced social behaviour, loss of coat grooming and partial hunched posture. These symptoms are expected to last 3-5 days, depending on the treatment. However, full recovery is expected to be achieved within 7-14 days. When un-immunized mice are infected with non-attenuated Salmonella strains, they will be killed within 5 days post infection, before developing severe clinical symptoms.
Injections are expected to cause local temporal pain, with no longer term effect.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For the mice on breeding and maintenance protocols (which takes up to 55% of the mice on this project) we expected the majority to experience sub-threshold (approximately 95%) a small number to experience mild severity (approximately 4%) and less than 1% to suffer moderate severity.
For the mice on experimental protocols (3, 4 and 5), we expected the majority (approximately 90%) to experience moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our studies directly investigate the complex interactions between immune cells and their physiological environment. Many of the processes we explore involve cell moving between organs, moving between different environment within minutes, processes that often depend on the intact blood and lymph flow.
A mammalian species is required to investigate the behaviour of immune cells in distinct anatomical niches, a dynamic and complex process that cannot be reproduced in vitro. For example, to study the migratory patterns of lymphocytes in the blood-rich compartments of the spleen, the intact blood flow must be maintained. Similarly, to investigate where and when a lymphocyte receives activation signals during an immune response the anatomical structure of the organ and its cellular composition must be preserved. To understand how cells cross in and out of restricted tissues (e.g. when entering or exiting to and from lymphoid organs), the intact entry and egress site of these structures and their connectivity to the circulatory fluids must be maintained. Notably, in many cases, the exact anatomical location and the cellular composition of niches are not known, therefore requiring direct approaches to discover them and study them.
Given that the adaptive immune system is an attribute restricted to vertebrates and reaches its full maturation in adults, addressing the scientific question in our studies requires the use of adult mice, with a fully mature immune system. In some cases, we may initially apply procedures on juvenile or embryos mice in order to induce specific changes that occur early during development and which subsequently affect the adult animal. However, the end point of such experiments will be explored in the adult animal.

Which non-animal alternatives did you consider for use in this project?

In some cases, cell culture systems may replace animal tissue in experiments that characterise the biochemical effects of specific genes; these approaches can complement in vivo studies. For example, we have recently established a collaboration with a group in Japan that allows us to use a cell line that can promote differentiation of specific stages of B cells in vitro. This system can replace and complement some of the in vivo procedures. In other cases, we use in vitro trans well analysis to explore the biochemical pathways that regulate cell migration.

Why were they not suitable?

Currently, we have limited understanding of the cellular composition, structural organisation and plasticity of most of the anatomical sites in which immunity is induced. There is therefore not enough knowledge to design artificial whole animal systems that will mimic the complexity of living organisms and allow us to explore how interactions with the tissue regulate immune responses.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of mice was estimated based on my extensive experience with the experimental approaches used in this proposal and the records of mice usage we have been keeping over the past 5 years.
These take into account both the statistical power of the different types of experiments involved, the various time points required for different assays and the frequencies by which multiple allele genotypes are generated in different crosses.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

1. **Appropriate use of statistics**:

The majority of our experiments produce data that can be analysed by pragmatic approaches such as test. We typically seek to uncover large differences in behaviour and function (~40%) and therefore do not normally need to use large numbers of animals to reach a statistically meaningful result in each experiment.
In most cases, animal numbers have been calculated based on our previous studies, assuming a standard deviation at the range of ~0.25 for imaging studies, and ~0.2 for most functional studies. Therefore, to obtain a statistically significant measurement (assuming one-tailed t test, significance level of <0.05 and the minimal acceptable power of 0.8), between 4-5 mice will be required to uncover differences of 40% (or more). Typically, 3 repeats will be sufficient to obtain statistically meaningful results.
When appropriate (e.g. when analysing histological sections), we use blinding to minimize biases.

2. **Minimise random variation and increase uniformity within and between experiments**. This is necessary to detect significant differences with minimal numbers of animals:

We use age and sex matching approaches to reduce variability between mice within experiments.
We only use fully backcrossed mice with the same genetic background.
We often co-house experimental animals in order to further increase uniformity between the different experimental groups.
When possible, we include intrinsic controls (e.g. using mixed bone marrow chimeras) to reduce the numbers of mice required per experiment by 50% and to further increase uniformity.

3. **Improved analysis methods**:

We develop new algorithms to improve statistical strength- Many of our studies are based on live imaging assays. These assays require multiple repeats due to a relatively high level of noise; an issue commercial software packages are unable to resolve. We have been working in collaboration with other groups to develop algorithms to overcome some of these limitations and extract more reliable data from each movie.

4. **Avoid experimental repetition**:

In some cases, we perform several experiments simultaneously, such that one cohort of control mice can be used as reference for more than one experimental question.
Similarly, we reduce experimental repetition and increase internal consistency (and therefore statistical strength)

by collecting all the relevant tissues from mice allowing us to compare different parameters within the same animals.
Frozen tissues are often archived to permit multi-factorial analysis without additional in vivo experiments (e.g. for using in histology).

5. Reducing number of donor mice:

In some cases, we use bone marrow chimeras and genetically engineered mouse models to generate mice in which specific lymphoid and non-lymphoid niches are highlighted endogenously, allowing live imaging of defined sites within living organs. This approach replaces the need to transfer large number of lymphocytes to achieve similar labelling of compartments, thus reduces the number of donors and provides a consistent system to address complex questions in vivo.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

1. Performing new experiments-

When performing a new assay, we first attempt to find labs that are routinely performing the assay and work with well-established protocols obtained from experienced researchers.
For novel assays, we first perform a pilot test to determine intra and inter group variations.

2. Optimised breeding strategy –

We review animal requirements frequently. I personally supervise this process.
We keep a careful documentation of the number and type of breeders to help organize the colony and ensure no unnecessary breeding is carried out. Our colony is managed directly by myself. I visit the mouse house very frequently and put a lot of efforts to ensure we do not generate unnecessary mice and use the pups born on our colony as best as we can.
We optimize breeding strategy by crossing homozygous breeder pairs when possible. Similarly, most transgenic animals are maintained as homozygous.
Embryos of strains that are not currently in use are frozen.
Some of our studies depend on complicated crosses that require expression of at least 3 alleles. In these cases, we typically use bone marrow chimeras to expand the desired genotype, such that one breeder is enough to obtain dozens of useful mice.

3. Sharing mice with other groups and resources-

When available, we obtain genetically modified mice from a relevant supplier or from colleagues. When specific lines do not exist, we generate them ourselves. We typically use external companies to make genetically altered mice, which is much more efficient and given that we are not experts in the area. Once we produce a new line and publish it, we make it available for the wider scientific community.
Our institute has an email list that allows us to distribute or obtain surplus mice, whenever they are available.

4. In vitro experiments-

1. Whenever possible, we use tissue culture and ex-vivo approaches to test our hypothesis.

2. In some cases, e.g. when studying dynamics of immune cell responses in niches in which cell behaviour does not depend on intact blood flow (such as the follicular regions in the spleen, lung or other peripheral sites) we use an explant instead of an intravital imaging approach. For this purpose, we have recently purchased a vibratome in the institute that allows gentle cutting of explant organs, maintaining the integrity of the tissue and have optimized a protocol to image explants successfully. Since multiple slices can be imaged from a single mouse, this approach also reduces the number mice required to obtain statistically meaningful information.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This proposal is limited to mice models. In this proposal, we will focus on experimental mouse models aiming to investigate basic mechanisms that regulate activation of adaptive immunity and generation of long-lasting antibody responses. To address these goals, we will use a range of approaches including immunisation with attenuated pathogens (e.g. such as attenuated vaccina virus which is used for vaccines), engineered proteins or responses and to test the mode of action of vaccines (such as the current anti-tuberculosis vaccine), infection models (e.g. influenza virus, to explore how antibodies against respiratory pathogens can be better induced and maintained). We will combine these approaches with cutting edge imaging techniques, which will allow us to obtain unique information about the dynamic of immune responses (how cells are able to respond accurately and quickly enough to invading pathogens).

Why can't you use animals that are less sentient?

Our studies focus on the regulation of adaptive immune responses, which have evolved in vertebrates, therefore excluding the usage of lower organisms. Because the adaptive immune system takes time to mature (e.g. marginal zone B cells take 8 weeks to develop in mice), using adult animals is necessary.
The mice we are using have to be alive during the experimental procedure. This is because we are studying mechanisms of cell trafficking via niches and organs that are not only complex but also often uncharacterized and therefore cannot be recapitulated in vitro (e.g. we search for the egress site from spleen, which currently remains an unknown anatomical structure). Furthermore, we depend on live animals because our studies explore responses that require cell trafficking between and within organs, a process that depends on intact blood and lymph circulation and tissue integrity.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We pay careful attention to animal husbandry and provide environmental enrichment and co-housing to avoid social isolation. For example, we keep careful track of weaning days to ensure that if a single male or females are due to be weaned, they are co-housed with other pups that are weaned on the same day from different lines.
We make every effort to reduce the number of procedures per animals and to minimize the discomfort involved during each procedure. For example, we previously amended the protocol to allow us to use implanted mini pumps and slow release pellets to reduce number of injections, in some experiments
We also minimize the suffering of animals through careful experimental design and by ensuring that the researcher performing the procedure is fully competent and understands the protocol and its limitations. For this reason, I discuss the aim and design of every in vivo experiment in the lab and I monitor routinely the competency level of researchers both by observing their performance as well as by evaluating the accuracy and consistency of their results.
We always aim to use the least painful substance and route of administration. For example, we have recently begun a scientific collaboration allowing us to use virion-like particles as a means to induce or re-activate local immune responses in the lung. This procedure causes no detectable adverse effects and so whenever possible, we use it instead of live viral infection.
When possible and necessary, we use anaesthetics to reduce temporary discomfort during a procedure. In these cases, animals are allowed to fully recover between the two restraint anaesthetics.
Our preferred choice of anaesthetic is inhalation, which we use whenever possible. Our institute's facility invested in new excellent Isoflurane equipment distributed by a local producer that worked with us to optimise it for our live imaging purposes. This equipment is highly reliable in ensuring the appropriate level of anaesthetic is delivered during surgical procedures and we now use it to fully replace injectable anaesthetic for our intravital imaging studies.
When surgery is involved, we use appropriate aseptic techniques, monitor the animals before during and after the procedure. Special attention is given to the husbandry of animals after surgery to monitor that they recover well.

When mice are subjected to treatments that may cause them moderate pain, a humane practice is exercised to limit the length of procedure to the shortest possible time.
When mice are infected with live pathogens, we aim to use the least virulent pathogenic strains, in order to reduce suffering. For example, we have obtained attenuated influenza strains that are capable of a single round of replication. We use these strains whenever possible, to prevent necessary suffering for the animals. Similarly, infection with Listeria will be primarily done using the attenuated Act A deficient strain, which significantly reduces the severity of adverse effects.
In all protocols, we follow clearly defined action points, monitoring schemes and humane end points to minimize suffering of animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will use our extensive experience as well as various online tools to calculate the number of mice for each experiment (e.g. using the NC3Rs Experimental Design Assistant (EDA) on-line tool). We will use ARRIVE guidelines for conducting and reporting our experiments for publication. If new or complicated statistical analysis is required, we will seek the advice of a statistician. Such help is readily available to us.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly attend welfare meetings and continue to use the NC3R website (https://www.nc3rs.org.uk) to follow up on news and updates. When introducing new treatments or approaches, we will seek the advice of experts in the field as well as that of the vet and NACWO regarding the latest relevant refinements.

Home Office

NON-TECHNICAL SUMMARY

45. Early behaviour and cognitive abilities in chickens (Gallus gallus)

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

predispositions, filial imprinting, chicks, controlled-rearing, cognitive development

Animal types	Life stages
Gallus gallus	neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand the developmental origins of early cognitive abilities using chicks of the

domestic fowl (*Gallus gallus*) as a model system. I plan to clarify how spontaneous preferences (predispositions) of inexperienced chicks affect/enhance learning, generalisation and social behaviour, and which is the role of the right and left brain hemispheres in cognitive development.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Due to ethical and practical constraints, the role of predisposed knowledge on cognitive traits such as learning and exploratory behaviour cannot be conveniently investigated in human infants, rodents and other species immature at birth, producing an important knowledge gap in understanding how the mind works and develops. Using chicks, that are precocial (hatch with a mature sensory and motor system) and are able to quickly learn through filial imprinting (a quick learning mechanism that is based on mere exposure and is in place at the beginning of life in precocial species such as chickens), we will clarify which are the building blocks of cognition in the first stages of life. In this way, we will obtain a better understanding of the cognitive abilities that support human cognitive development, learning and social behaviour. This project is important to understand healthy cognitive development. Given that these abilities are compromised in neurodevelopmental disorders such as autism, and that chicks are a model also for neurodevelopmental disorders, this research has also clinical relevance; moreover, it can lead to the development of better biologically-inspired artificial intelligence.

What outputs do you think you will see at the end of this project?

Predispositions: we will clarify which features are preferred by chicks in terms of colour, shape, motion dynamics, social engagement.
Generalisation and learning: we will clarify whether predispositions (spontaneous preferences) enhance learning and generalisation in new situations. We will determine the minimal experience required for spontaneous generalisation.
Our data will inform experts in artificial networks on effective strategies for generalisation.
We will integrate the information gained on chicks' performance with artificial intelligence models to advance the understanding of animal minds, cognitive and behavioural development and artificial intelligence.
Publications: our findings and models will be published on peer-reviewed publications accessible to the scientific community and the public. To reach a cross-disciplinary audience, we will make our findings available to the scientific community and public through open servers before experts review and target interdisciplinary and open access expert-reviewed journals.
Data will be made available on public repositories and presented at international and national conferences/webinars.

Who or what will benefit from these outputs, and how?

The problem of the origins of knowledge has been investigated for centuries, with implications that span from philosophy to linguistics, psychology, neuroscience and artificial intelligence.
Developmental psychologists will be informed on the early mechanisms of generalisation with visual stimuli, paving the way to studies in different modalities and domains, including language, and in human learning and education. The potential of the chick as a model relevant for humans is shown by the fact that previous experiments conducted by my research group and other groups have shown that the same/similar deficits/delays present in children at high risk of autism are present in chicks exposed to substances that increase the risk of autism in humans (such as valproic acid). The proposed behavioural work, hence, builds on previous data that suggest a benefit of our studies not only to better understand the behaviour of chicks but also to understand human behaviour.
Thanks to our work, researchers interested in the neurobiological basis of spontaneous preferences and generalisation will have an established animal model system and setting to investigate the responsiveness of different brain areas using methods that require refined behavioural methods and few animals.

Our findings and models are expected to move artificial intelligence towards general intelligence. This can have a significant socio-economic impact in industrial applications (e.g. artificial intelligence, robotics, automation) and for the everyday life of the general public.

How will you look to maximise the outputs of this work?

To reach a cross-disciplinary and broad audience, we will make our findings available to the scientific community and the public through: open repositories; interdisciplinary and open access peer-reviewed journals; presentations and posters at international and national conferences/webinars. Data and protocols will be made available on public repositories.

Species and numbers of animals expected to be used

- Domestic fowl: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

To study the cognitive abilities and behaviours described in this project, we need to work with live animals, given that there is little or no evidence available on the topic. Only intact animals will allow to identify valid behavioural responses.
We will use newborn domestic chicks (*Gallus gallus*) because we are interested in shedding light on the early cognitive abilities and behavioural skills, at the beginning of life, when little experience is available.
It has not been possible to identify animal models that have a lower neurological development than the chick, that with a comparable sensory and cognitive development provide the same behavioural complexity in such as precocial developmental phase, and that have mechanisms of learning as efficient as those present in young chicks in terms of speed and flexibility, and that is an established model in the previous literature.
The choice of using domestic chicks (instead for instance of rodents) is well supported by virtue of their peculiar maturity at hatching. Chicks in fact hatch with a mature sensory and motor system. This facilitates testing newborn animals, fully controlled until the moment of test. This reduces the need of external carers that would influence the results, and minimises the amount of time that chicks spend in the laboratory setting. The fact that chicks can establish social attachment for artificial objects and not only for the hen, also helps our research and reduces the impact of social restrictions.

Typically, what will be done to an animal used in your project?

We will run behavioural experiments, exposing young individual animals to determine visual/acoustic/tactile stimuli and observing their approach preferences and other measures of interaction (e.g. pecking). Chicks will be exposed only to the stimuli delivered experimentally in a restricted/controlled space, and their social companions will be artificial tridimensional objects or computer displays.

Some animals will undergo behavioural tests that will help identify the different function of the right and left brain hemisphere in learning and other behaviours. To this aim, one eye will be temporarily covered with an eye patch, such that only one hemisphere receives visual input at a time in relevant experiments.

What are the expected impacts and/or adverse effects for the animals during your project?

Only healthy chicks will be tested.
Based on the previous literature and on the principal investigator's experience with this animal model and procedures, only temporary mild adverse effects are expected. One possible source of mild suffering is the reduction of space and environmental and social enrichment in the controlled-rearing environment, since animals may be housed with artificial partners (e.g. a cube, a cylinder, a computer display) rather than in groups with conspecifics, in basic accommodation with minimal inclusions (food, water), to avoid chicks imprinting on any items that would affect experimental outcomes.
However, this is well tolerated and no long-term adverse effects are regularly observed. We will monitor the health parameters and behavioural responses of chicks to prevent distress. Any runts or those that show signs of ill health that cannot be quickly ameliorated will be humanely killed. Care will be taken to ensure those under experimental conditions can readily access food and water at all times.
Another source of potential mild distress is the covering of one eye with an eye-patch. The discomfort will be reduced avoiding to touch the eye with sticky material. This procedure has been used for decades and it is well tolerated by chicks. No long-term adverse effects have been observed. Shall for any reason a chick show signs of sustained distress in response to the eye-patch, it won't be tested and the patch will be removed to prevent further distress.
When tested in a new apparatus, a few animals might be distressed by the detachment from the home cage. In case the chick shows sign of distress (pecking at arena wall, trying to jump out while producing distress calls) for more than 2 minutes, the test will be aborted and the animal located back in its home cage. In these cases, while moving the chick back to its home cage, the experimenter will comfort the chick with a gentle touch.
The overall health of the flock will be checked also by looking at the overall mortality rate, to make sure it does not exceed the spontaneous mortality rate observed in the first 2 weeks of age.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The maximum severity of experience for animals on the project will be mild, because any pain or suffering experienced by an animal will only be slight, transitory and minor. We expect that most animals may experience a subthreshold severe experience.
100% of animals will be controlled-reared with limited exposure to environmental and/or social enrichment. We expect adverse effects in less than 10% of the animals.
50% of animals will be tested in a new apparatus. We expect adverse effects in less than 10% of the animals.
10% of animals will be tested with an eye-patch. We expect adverse effects in less than 10% of the animals.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Ethical and practical limitations prevent us to keep and test humans in a controlled-rearing environment. The

investigation of cognitive abilities and behaviours described in this project requires to work with live animals, given that there is little or no evidence available on the topic. Only intact animals will allow us to identify valid behavioural responses.
It has not been possible to identify animal models other than the chicks with a lower neurological development that, with a comparable degree of development at the sensory and cognitive level, provide the same behavioural complexity in such an early developmental phase and that have mechanisms of learning as efficient as filial imprinting in the terms of speed and flexibility, and so well studied in the previous literature.
The choice of using the domestic chick is well supported also by virtue of its peculiar precocial features, that minimise the duration of time spent in the laboratory setting, ease of experimental handling, similarity to human responses.

Which non-animal alternatives did you consider for use in this project?

In-silico models are not suitable given the little knowledge available on the topic. For this reason, the domestic chick is widely used to address questions about early cognition.
This project will provide evidence to develop artificial intelligence that in the future could substitute or reduce animal use.

Why were they not suitable?

We are interested in the building blocks of animal cognition and behaviour, for this reason only intact living animals will allow to identify valid behavioural responses.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We plan to test the minimum number of animals that enables to draw valid statistical conclusions: 40-50 animals depending on the procedure. Animals will be tested only once because we want to identify the role of a particular experience regime at a time.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Based on power calculations on the previous literature and pilot experiments we have calculated that each experimental condition will require 40-50 animals, depending on the experimental condition.

What measures, apart from good experimental design, will you use to optimise the number of animals you

plan to use in your project?

Random behavioural variability will be reduced by careful and standardized handling of the eggs (e.g. egg development synchronized by locating eggs in a fridge before starting incubation, identical temperature and humidity settings) and of the chicks before and during the test. Moreover, the incubation room, maintenance room and testing room will be climatically standardized.
We have run pilot studies that confirm the suitability of 40-50 animals to detect a medium size effect which will be of statistical significance.
Measurement variability will be reduced using automated behavioural tracking based on machine learning.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use newly-hatched domestic chicks (*Gallus gallus*), that present several advantages in terms of reducing the suffering in the required controlled-rearing procedures:

- they are mature and can take part in the experiments soon after hatching, thus reducing the time spent in the laboratory

- they can become attached to the simple artificial objects required by this study and do not require care from other living animals

- because in birds the right and left hemispheres have few connections, lateralised functions can be studied using non-invasive eye patches

- the literature shows that the suggested procedures are well tolerated

Why can’t you use animals that are less sentient?

We have not identified animal models with a lower neurological development that, with a comparable degree of development at the sensory and cognitive level, provide the same behavioural complexity in such as precocial developmental phase and that have mechanisms of learning as efficient as filial imprinting in the terms of speed and flexibility, and so well studied in the previous literature.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will carefully monitor the health and behaviour of chicks. The use of automated behavioural tracking enables us to carefully inspect animal behaviour, reduces the number of animals required in the tests and provides data that can be subsequently analysed.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Experimental protocols on the procedures suggested in this project are shared by the scientific community and

have already been published in open source journals. We keep up to date with the relevant scientific literature to refine our procedures.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I am regularly in touch with the officers at my establishment, that organise outreach/instruction events, and with colleagues that work with poultry. I also check the NC3R website and I keep up to date with the literature. Moreover, I am a member of the Association for the Study of Animal Behaviour and I participate in its activities, as well as to the Animal Welfare Research Network.

Home Office

NON-TECHNICAL SUMMARY

46.Early Diagnosis and Therapy through the Eye

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

retinal neurodegeneration, ocular surface disorder, disease models, imaging, therapy

Animal types	Life stages
Mice	adult
Rats	adult
Rabbits	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To characterise disease models with retinal neurodegeneration or with dry eye in order to develop therapeutic strategies for the treatment of ocular diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Retinal neurodegeneration (RN) is the major cause of irreversible blindness worldwide. There is currently no early diagnosis and no cure. The retinal degenerative condition occurs not only in eye diseases, such as glaucoma and age-related macular degeneration (AMD) but also in neurodegenerative disorders, including Alzheimer's (AD) and Parkinson's (PD). Dry eye disease (DED) is a multifactorial ocular surface disease characterised by symptoms of discomfort, irritation, and visual disturbance. DED is a very common condition with a high prevalence among the elderly and has no cure. In monitoring the basic mechanisms of retinal neuronal loss in RN and ocular surface abnormality in DED, this work aims to shed light on poorly understood ocular diseases and characterise the ocular manifestation of systemic conditions. These approaches would allow us to develop new strategies for treating widespread diseases thus prevent or delay the onset of blindness. The wider implications would impact on any conditions associated with neuronal loss and ocular surface disorder, where our findings can help to enhance the clinical diagnosis and refine the therapeutic intervention.

What outputs do you think you will see at the end of this project?

- This work is expected to shed light on poorly understood ocular diseases and to identify and characterise the ocular manifestation of systemic diseases.
- Our findings are expected to potentially help to enhance the clinical diagnosis and refine the therapeutic intervention.
- The primary expected benefit is the publication of new therapeutic strategies for retinal neurodegenerative diseases and dry eye.

Who or what will benefit from these outputs, and how?

Throughout the life of this project, data produced will be presented at national and international conferences and published in academic journals. The new information will provide a new understanding of the mechanisms behind retinal neuronal death and identify new strategies to reduce or halt neuronal loss in neurodegenerative diseases. We will also promote and publish any refinements or best practices we identify during this project. We will use post-mortem tissue to correlate ocular changes elsewhere in the body to make maximum use of a single animal.

In the medium-term, the pharmaceutical industry will be interested in potential novel therapeutic targets we identify.

The long-term potential benefits of this study are that data generated may have far-reaching implications for the treatment of neurodegenerative diseases, both in humans, benefitting patients and clinicians by contributing to the development of effective neuroprotective therapies, which will ultimately reduce the economic and health

burden caused by the devastating conditions.

How will you look to maximise the outputs of this work?

We will collaborate with other researchers and share new data and knowledge as well as unsuccessful approaches.

Species and numbers of animals expected to be used

- Mice: 3000
- Rats: 2000
- Rabbits: 200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rats and mice are the main species to be used in this project because the rodent is the lowest vertebrate group with many similarities to human tissues in structure and function. We now have a great experience with these models in imaging nerve cell death in the back of the eye and making different disease models.

We will only use transgenic rodent models that have been closely linked to human disease. Transgenic animals provide a chronic model of disease that is not easily achievable in surgically or substance induced models, such as mouse models of Alzheimer's and Parkinson's disease.

However, to test the safety of the drugs and to develop new drug administration routes for translating the treatments to humans in the future, we would like to use the rabbit as it is a well-known and well-studied model of eye disease and is particularly important for its similarity to the human eye. Specifically, we will use New Zealand rabbits.

The majority of these animals used in this project will be young adults, however, a small number of ageing animals will be included to study ageing changes and treatment efficacy, such as transgenic Alzheimer's and Parkinson's mice.

Typically, what will be done to an animal used in your project?

Animals will undergo one procedure to make a disease model and will receive neuroprotective substances or vehicle via one or two administration routes, and will be assessed for retinal structural and/or functional changes and therapeutic efficacy. At the end of the experiment, animals will be killed by a Schedule 1 method or perfusion fixation.

What are the expected impacts and/or adverse effects for the animals during your project?

The expected adverse effects include corneal cloudy, some degree of vision loss, post-surgical pain, weight loss, and weakness. Because disease models will be induced in one eye and left the other eye intact, corneal

cloudy and vision loss will occur in one eye only, which would not affect overall animal welfare. Post-surgical pain may last less than 24 hours and topical and systemic analgesia will be administered. Weight loss and weakness may occur in some disease models, such as diabetic retinopathy and Parkinson's disease. To reduce the duration of these adverse effects, animals will be kept for a shorter period of time as possible, for example, diabetic animals for 4-24 weeks and
Parkinson's for 8 weeks. Animals will be monitored and scored daily for clinical signs, and any animal who has a weight loss of more than 15% will be killed by a Schedule 1 method.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Although the severity of this project has been set up as moderate, the majority of animals will experience mild procedures. A small proportion of animals will be expected to experience moderate, such as surgically-induced glaucoma models and substance-induced diabetics. The proportion of animals who may have moderate severity in this project as a whole will be less than 25% in mice and rats, and 5% in the rabbits.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This work relies on the assessment of processes in the living being – it is not possible to use cell culture or test-tube, as studying whole-body conditions is essential. There is no alternative to using live animals to answer our scientific questions.

Which non-animal alternatives did you consider for use in this project?

For partial replacement, cell culture methods are useful to assess drug safety, to screen drug candidates, and to optimise dosages before testing them in animals. This has been used in our previous PPLs and will be continually used in this project.

Why were they not suitable?

However, cell culture or test-tube methods do not allow us to directly apply to patients. It is not possible to reproduce the highly complex anatomical structure of the eye and brain in cell culture or test tube. Moreover, our studies are exploring the use of real-time retinal nerve cell death and apoptosis measurements and their correlation with disease and treatment. This cannot be done in tissue culture models where the environment can never be completely replicated.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our study is designed on the basis of pilot studies and previous research, to optimise the information gained with the minimal use of animals, with good scientific practice.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I have recently attended a course of the NC3R's Experimental Design Assistant (EDA). The useful online tools show how to design the randomisation and blinding of the experiment and sample size calculation, in order to produce robust and reproducible data with a minimum of animal use, such as randomised block experimental design. This accounts for the influence of variables and addresses sources of bias, appropriate controls, and efficient use of statistics to ensure that the data from every animal is utilised to its full potential.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

A major advance in our laboratory research is our use of in vivo techniques for disease assessment e.g., cell death imaging and electrophysiology. This allows the collection of serial data so the same animal can be tracked over time, and recent research has enabled us to reduce the number of animals used compared with previous applications. Furthermore, the parallel cell culture studies will enhance the investigation of neuroprotective strategies, highlighting appropriate agents and dosages.

In addition, to optimise the number of animal use, we will breed animals efficiently to make sure not to breed more than we need. We will collaborate with other PPL holders, who have breeding protocols for genetically modified animals. We will continue to use pilot studies and computer modelling as well as share tissues with others. For example, we have recently obtained mouse eyes with multiple sclerosis by collaborating with another group.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats and mice are the main species to be used in this project because the rodent is the lowest vertebrate group with many similarities to human tissues in structure and function. However, to test the safety of the neuroprotective agents and to develop novel drug delivery for translating the treatments to humans in the future, we would like to use the rabbit as it is a well-known and well-studied model of ocular disease and is particularly appreciated for its similarity to the human eye. Specifically, we will be studying drug delivery and islet cell transplantation in NZW rabbits. All models chosen in the new project are the most established and accepted models that involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress, or lasting harm, and are the most likely to produce satisfactory results. These models have either been established by us, and covered in previous PPLs, or are established by other experts in our university.

Why can't you use animals that are less sentient?

The animal models with retinal neurodegeneration are to mimic human diseases that occur in adulthood and mostly in ageing. So, it would not be suitable to use the immature life stage of animals. Rodent models of retinal disease have been well established and widely used worldwide. The majority of animals in the project will be studied over time to determine structural and functional changes and to assess therapeutic efficacy. However, some animals will be considered to be terminally anaesthetised where it is possible.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimise stress, animals will be handled and trained before procedures, such as clicker training (https://www.jove.com/video/58511/using-clicker-training-social-observation-to-teach-rats-to) and tickling (https://www.jove.com/video/57190/tickling-a-technique-for-inducing-positive-affect-whenhandling-rats); less harmful models will be used, such as using Akita females instead of males; and less invasive methods will be used, such as use ear snips rather than tail tips for genotyping.

Beware of potential adverse effects of the procedures before being performed. To minimise any adverse effects, animals will be carefully monitored for any signs of distress and pains after procedures, with post-operative care and pain management.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure experiments are conducted in the most refined way, I will follow the best practice guidance, such as The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) for experimental design, and The ARRIVE (Animal Research: Reporting of In Vivo Experiments) for publication of results.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I have registered and attended the course of the 3Rs, and will stay informed about advances in the 3Rs by visiting the NC3Rs website regularly and reading newsletters, and by attending national and international conferences.

Home Office

NON-TECHNICAL SUMMARY

47.Early Diagnosis and Therapy through the Eye

Project duration

5 years 0 months

Project purpose

- (c) Basic research
- (d) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

retinal neurodegeneration, ocular surface disorder, disease models, imaging, therapy

Animal types	Life stages
Mice	adult
Rats	adult
Rabbits	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To characterise disease models with retinal neurodegeneration or with dry eye in order to develop therapeutic strategies for the treatment of ocular diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Retinal neurodegeneration (RN) is the major cause of irreversible blindness worldwide. There is currently no early diagnosis and no cure. The retinal degenerative condition occurs not only in eye diseases, such as glaucoma and age-related macular degeneration (AMD) but also in neurodegenerative disorders, including Alzheimer's (AD) and Parkinson's (PD). Dry eye disease (DED) is a multifactorial ocular surface disease characterised by symptoms of discomfort, irritation, and visual disturbance. DED is a very common condition with a high prevalence among the elderly and has no cure. In monitoring the basic mechanisms of retinal neuronal loss in RN and ocular surface abnormality in DED, this work aims to shed light on poorly understood ocular diseases and characterise the ocular manifestation of systemic conditions. These approaches would allow us to develop new strategies for treating widespread diseases thus prevent or delay the onset of blindness. The wider implications would impact on any conditions associated with neuronal loss and ocular surface disorder, where our findings can help to enhance the clinical diagnosis and refine the therapeutic intervention.

What outputs do you think you will see at the end of this project?

- This work is expected to shed light on poorly understood ocular diseases and to identify and characterise the ocular manifestation of systemic diseases.
- Our findings are expected to potentially help to enhance the clinical diagnosis and refine the therapeutic intervention.
- The primary expected benefit is the publication of new therapeutic strategies for retinal neurodegenerative diseases and dry eye.

Who or what will benefit from these outputs, and how?

Throughout the life of this project, data produced will be presented at national and international conferences and published in academic journals. The new information will provide a new understanding of the mechanisms behind retinal neuronal death and identify new strategies to reduce or halt neuronal loss in neurodegenerative diseases. We will also promote and publish any refinements or best practices we identify during this project. We will use post-mortem tissue to correlate ocular changes elsewhere

in the body to make maximum use of a single animal.

In the medium-term, the pharmaceutical industry will be interested in potential novel therapeutic targets we identify.

The long-term potential benefits of this study are that data generated may have far-reaching implications for the treatment of neurodegenerative diseases, both in humans, benefitting patients and clinicians by contributing to the development of effective neuroprotective therapies, which will ultimately reduce the economic and health burden caused by the devastating conditions.

How will you look to maximise the outputs of this work?

We will collaborate with other researchers and share new data and knowledge as well as unsuccessful approaches.

Species and numbers of animals expected to be used

- Mice: 3000
- Rats: 2000
- Rabbits: 200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rats and mice are the main species to be used in this project because the rodent is the lowest vertebrate group with many similarities to human tissues in structure and function. We now have a great experience with these models in imaging nerve cell death in the back of the eye and making different disease models.

We will only use transgenic rodent models that have been closely linked to human disease. Transgenic animals provide a chronic model of disease that is not easily achievable in surgically or substance induced models, such as mouse models of Alzheimer's and Parkinson's disease.

However, to test the safety of the drugs and to develop new drug administration routes for translating the treatments to humans in the future, we would like to use the rabbit as it is a well-known and well-studied model of eye disease and is particularly important for its similarity to the human eye. Specifically, we will use New Zealand rabbits.

The majority of these animals used in this project will be young adults, however, a small number of ageing animals will be included to study ageing changes and treatment efficacy, such as transgenic Alzheimer's and Parkinson's mice.

Typically, what will be done to an animal used in your project?

Animals will undergo one procedure to make a disease model and will receive neuroprotective substances or vehicle via one or two administration routes and will be assessed for retinal structural and/or functional changes and therapeutic efficacy. At the end of the experiment, animals will be killed by a Schedule 1 method or perfusion fixation.

What are the expected impacts and/or adverse effects for the animals during your project?

The expected adverse effects include corneal cloudy, some degree of vision loss, post-surgical pain, weight loss, and weakness. Because disease models will be induced in one eye and left the other eye intact, corneal cloudy and vision loss will occur in one eye only, which would not affect overall animal welfare. Post-surgical pain may last less than 24 hours, and topical and systemic analgesia will be administered. Weight loss and weakness may occur in some disease models, such as diabetic retinopathy and Parkinson's disease. To reduce the duration of these adverse effects, animals will be kept for a shorter period of time as possible, for example, diabetic animals for 4-24 weeks and
Parkinson's for 8 weeks. Animals will be monitored and scored daily for clinical signs, and any animal who has a weight loss of more than 15% will be killed by a Schedule 1 method.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Although the severity of this project has been set up as moderate, the majority of animals will experience mild procedures. A small proportion of animals will be expected to experience moderate, such as surgically-induced glaucoma models and substance-induced diabetics. The proportion of animals who may have moderate severity in this project as a whole will be less than 25% in mice and rats, and 5% in the rabbits.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This work relies on the assessment of processes in the living being – it is not possible to use cell culture or test-tube, as studying whole-body conditions is essential. There is no alternative to using live animals to answer our scientific questions.

Which non-animal alternatives did you consider for use in this project?

For partial replacement, cell culture methods are useful to assess drug safety, to screen drug candidates, and to optimise dosages before testing them in animals. This has been used in our previous PPLs and will be

continually used in this project.
Why were they not suitable?

However, cell culture or test-tube methods do not allow us to directly apply to patients. It is not possible to reproduce the highly complex anatomical structure of the eye and brain in cell culture or test tube. Moreover, our studies are exploring the use of real-time retinal nerve cell death and apoptosis measurements and their correlation with disease and treatment. This cannot be done in tissue culture models where the environment can never be completely replicated.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our study is designed on the basis of pilot studies and previous research, to optimise the information gained with the minimal use of animals, with good scientific practice.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I have recently attended a course of the NC3R's Experimental Design Assistant (EDA). The useful online tools show how to design the randomisation and blinding of the experiment and sample size calculation, in order to produce robust and reproducible data with a minimum of animal use, such as randomised block experimental design. This accounts for the influence of variables and addresses sources of bias, appropriate controls, and efficient use of statistics to ensure that the data from every animal is utilised to its full potential.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

A major advance in our laboratory research is our use of in vivo techniques for disease assessment e.g., cell death imaging and electrophysiology. This allows the collection of serial data so the same animal can be tracked over time, and recent research has enabled us to reduce the number of animals used compared with previous applications. Furthermore, the parallel cell culture studies will enhance the investigation of neuroprotective strategies, highlighting appropriate agents and dosages.

In addition, to optimise the number of animal use, we will breed animals efficiently to make sure not to breed more than we need. We will collaborate with other PPL holders, who have breeding protocols for genetically modified animals. We will continue to use pilot studies and computer modelling as well as share tissues with others. For example, we have recently obtained mouse eyes with multiple sclerosis by collaborating with another group.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats and mice are the main species to be used in this project because the rodent is the lowest vertebrate group with many similarities to human tissues in structure and function. However, to test the safety of the neuroprotective agents and to develop novel drug delivery for translating the treatments to humans in the future, we would like to use the rabbit as it is a well-known and well-studied model of ocular disease and is particularly appreciated for its similarity to the human eye. Specifically, we will be studying drug delivery and islet cell transplantation in NZW rabbits. All models chosen in the new project are the most established and accepted models that involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress, or lasting harm, and are the most likely to produce satisfactory results. These models have either been established by us, and covered in previous PPLs, or are established by other experts in our university.

Why can't you use animals that are less sentient?

The animal models with retinal neurodegeneration are to mimic human diseases that occur in adulthood and mostly in ageing. So, it would not be suitable to use the immature life stage of animals. Rodent models of retinal disease have been well established and widely used worldwide. The majority of animals in the project will be studied over time to determine structural and functional changes and to assess therapeutic efficacy. However, some animals will be considered to be terminally anaesthetised where it is possible.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimise stress, animals will be handled and trained before procedures, such as clicker training (https://www.jove.com/video/58511/using-clicker-training-social-observation-to-teach-rats-to) and tickling (https://www.jove.com/video/57190/tickling-a-technique-for-inducing-positive-affect-whenhandling-rats); less harmful models will be used, such as using Akita females instead of males; and less invasive methods will be used, such as use ear snips rather than tail tips for genotyping.

Beware of potential adverse effects of the procedures before being performed. To minimise any adverse effects, animals will be carefully monitored for any signs of distress and pains after procedures, with post-operative care and pain management.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure experiments are conducted in the most refined way, I will follow the best practice guidance, such as The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) for experimental design, and The ARRIVE (Animal Research: Reporting of In Vivo Experiments) for publication of results.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I have registered and attended the course of the 3Rs, and will stay informed about advances in the 3Rs by visiting the NC3Rs website regularly and reading newsletters, and by attending national and international conferences.

Home Office

NON-TECHNICAL SUMMARY

48. Ecotoxicity Studies with Amphibians

Project duration

5 years 0 months

Project purpose

- (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Endocrine, Disruptor, Xenopus, XETA, Chemicals

Animal types	Life stages
Xenopus	adult, pregnant, neonate, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of the project is to assess the interactions of a range of chemical types (industrial chemicals, agrochemicals, pharmaceuticals, biocides and microbial pesticides) with the endocrine systems of Xenopus tadpoles, Eleutheroembryos or transgenic Eleutheroembryos, such that the hazardous properties of these substances with respect to their ecotoxicological properties can be assessed. These properties are a fundamental requirement of the risk assessment process for such substances.
A retrospective assessment of these aims will be due by 21 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The data obtained in these tests are submitted to regulatory authorities to inform decision-making processes and, where appropriate, satisfy the governmental regulatory requirements that are necessary to gain product registration or to assess the risk and impact posed to the natural environment or human health by the use of chemicals, agrochemicals or pharmaceuticals. Ecotoxicology studies in general are designed to assess the likely impact on populations of plants and animals, and to identify ecologically benign concentrations.

What outputs do you think you will see at the end of this project?

The assessment of the risk posed by new and existing chemicals and waste materials to the natural environment continues to be an important international issue for industry, governments and the public alike. Synthetic chemical substances will inevitably enter the natural environment as a result of their use and disposal in industrial and domestic environments. Ecotoxicology studies are designed to assess their likely impact on natural populations of plants and animals, and to identify ecologically benign concentrations.
The main aim of the studies conducted within this project is to identify substances that may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis in amphibians, specifically *Xenopus laevis*. This is an important assay as amphibian metamorphosis is a thyroid dependent process, which is well studied and is the only assay that detects thyroid activity in an animal undergoing morphological development.

The studies are designed to evaluate a number of endpoints, including survival, developmental stage and associated morphological features, body wet weight and thyroid histology.
The use of Xenopus Eleutheroembryonic Thyroid Assay (XETA) will allow detection of potential modulations of thyroid activity induced by a range of test chemicals.

Who or what will benefit from these outputs, and how?

There are many concerns that environmental levels of specific chemicals interacting with the endocrine system (the oestrogen, androgen or thyroid hormone) may cause adverse effects in both humans and wildlife populations and have wider implications for both human and ecosystem health. Aquatic organisms have been identified as the most convincing examples of evidence for the impact that potential endocrine disrupting chemicals can have on animal health. These concerns have led to the revision and development of guidelines in order to facilitate screening and testing of these potential endocrine disruptors.
The data obtained in these tests are submitted to regulatory authorities to inform decision-making processes and, where appropriate, satisfy the governmental regulatory requirements that are necessary to gain product registration or to assess the risk and impact posed to the natural environment or human health by the use of chemicals, agrochemicals or pharmaceuticals. Ecotoxicology studies in general are designed to assess the likely

impact on populations of plants and animals, and to identify ecologically benign concentrations.

How will you look to maximise the outputs of this work?

All of the studies conducted in this facility with aquatic species are bound by confidentiality agreements and unless contracted to provide support through the registration process, the testing facility does not normally receive information regarding the progression of a substance through to marketing authorization. It is not possible therefore to identify the number of substances tested in the facility that have gained marketing authorization or product registrations. Success in this programme of work is not only associated with the successful registration of a substance but also the refinement of risk assessments, and the derivation of risk-reduction and toxicity mitigation strategies. Another important role of studies in this programme is the identification of substances with unacceptable effects or safety margins.

Species and numbers of animals expected to be used

- Xenopus: 50 (Adults), 13500 (tadpoles), 36,000(eleutheroembryos)

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult *Xenopus laevis* are required in order to produce eggs for the acute and definitive tests for the 21 day metamorphosis assay.
For this assay to be assessed correctly it is essential that a life stage is used that is going through the process of metamorphosis so that developmental end points can be assessed. Amphibian metamorphosis is a thyroid dependent process and is the only assay that detects thyroid activity in an animal undergoing morphological development.

Adult Xenopus laevis may also be used in order to produce eggs/ Eleutheroembryos for the XETA range-finding assay. Eleutheroembryos or transgenic Eleutheroembryos at stage NF45 are used for the XETA range-finder or definitive test respectively as this assay requires embryos at a specific developmental stage in order for this assay to work successfully. The definitive test requires the use of transgenic Eleutheroembryos as the fluorescence of each organism needs to be quantified at the end of the test.

Typically, what will be done to an animal used in your project?

21 day metamorphosis assay:

In order to conduct the aquatic toxicity tests using tadpoles at a specific morphological stage (Stage 51), the production of eggs from breeding adult *Xenopus laevis* held in the laboratory must be induced. All tadpoles exposed in the metamorphosis study must originate from the same spawning event, and therefore sufficient numbers of eggs are required. Approximately 12 hours before the required egg collection, injections of hCG (human Chorionic Gonadotrophin) are given, and the breeding pairs are then allowed to perform natural mating and egg laying procedures.
In order to conduct the assay Animals will be transferred from holding tanks to test vessels, transfer to fresh test media (where applicable) in all animals.
The developmental stage of the animals is determined using a binocular dissection microscope to ensure that

stage 51 tadpoles are utilized during the test. A selection of animals (approximately 20) may also be measured and a mean whole body length for this group of animals determined, minimum and maximum limits for the whole body length of experimental animals can be set by allowing a range of the mean value ± 3 mm.
There is a minimum of three test concentrations and a clean water control (and vehicle control if necessary). Separate groups (typically 80 tadpoles over 4 replicates per concentration) of stage 51 Tadpoles will be exposed concurrently to varying concentrations of the test item for a period of up to 21 days under semi-static or continuous flow test conditions.
Animals are observed at least daily. Where signs of toxicity are seen, the frequency of observations will be increased. At each observation time, animals that are considered likely to die or are showing symptoms of exposure that represent a significant departure from the animal's normal state of health or well-being will be identified and humanely killed by an appropriate non-schedule 1 method at the designated establishment. The tadpoles will be given an overdose of anaesthetic (eg MS222) for a period of at least 2 hours. The absence of response to a physical stimulus will be confirmed followed by permanent removal from the aqueous environment prior to immersing them in a suitable fixative (Davidsons solution or a suitable alternative). All humane kills are recorded.
Food will provided throughout the test, the type and amount of feeding will be dependent on the life stage of the larvae, whilst minimising the surplus that will be removed throughout the study.
A selection of tadpoles will be humanely killed and sampled once during the study, this will be conducted (e.g. on Day 7) by a non-schedule 1 method at the designated establishment. The tadpoles will be given an overdose of anaesthetic (e.g. MS222) for a period of at least 2 hours. The absence of response to a physical stimulus will be confirmed followed by permanent removal from the aqueous environment prior to immersing them in a suitable fixative (Davidsons solution or a suitable alternative).
At the end of the exposure period (e.g. Day 21) surviving tadpoles will be humanely killed by a nonschedule 1 method at the designated establishment. The tadpoles will be given an overdose of anaesthetic (eg MS222) for a period of at least 2 hours. The absence of response to a physical stimulus will be confirmed followed by permanent removal from the aqueous environment prior to immersing them in a suitable fixative (Davidsons solution or a suitable alternative) and all appropriate end-points determined.

Xenopus Eleutheroembryonic Thyroid Assay (XETA):

In order to conduct the assay Eleutheroembryos will be transferred from holding tanks to test vessels and transferred to fresh test media (where applicable).
The developmental stage of the Eleutheroembryos is determined using a binocular dissection microscope to ensure that stage 45 Eleutheroembryos are utilized during the test.
Eleutheroembryos between developmental stages NF45 (beginning of the test) and NF47 (end of the test) are used for this test. They are not fed before or during the test as yolk is still present in the intestine from stage NF45 to stage NF47 and is used as the source of energy for the development of the eleutheroembryo (Nieuwkoop and Faber, 1994).
There is a minimum of three test concentrations (+/- triiodothyronine (T3) 3.25 µg/L)) plus relevant control groups (Test media, Triiodothyronine (T3) and Thyroxine (T4)) (and vehicle control if necessary). Separate groups (typically 20 Eleutheroembryos over 2 replicates per concentration) of stage 45 Eleutheroembryos will be exposed concurrently to varying concentrations of the test item for a period of approximately 72 hours under semi-static or continuous flow test conditions. Three runs will be conducted per test.
Eleutheroembryos are observed at least daily. Where signs of toxicity are seen, the frequency of observations will be increased. At each observation time, Eleutheroembryos that are considered likely to die or are showing symptoms of exposure that represent a significant departure from the normal state of health or well-being will be identified and humanely killed by an appropriate non-schedule 1 method at the designated establishment. When both the acute and XETA studies have been completed, all remaining Eleutheroembryos are humanely killed by a non-schedule 1 method at the designated establishment. The Eleutheroembryos will be given an overdose of anaesthetic (eg MS222) for a period of up to 45 minutes (as specified in the test guideline) followed by permanent removal from the aqueous environment. All Eleutheroembryos will then be frozen (-18˚C) prior to destruction by incineration. (AC).
The fluorescence of each organism is quantified after 72 ± 2 h of exposure.

What are the expected impacts and/or adverse effects for the animals during your project?

Transfer of animals from holding tanks and transfer to fresh test media (where applicable) will cause mild stress in all animals. The discomfort will be transient in the majority of animals (>90%) and no additional action will be required.

It is anticipated that only transient minor discomfort should occur in the adult animals during injection procedures. Reactions to the gonadotrophin injected are not expected.

Due to the inherent nature of LC_{50} testing deaths and adverse clinical signs (e.g. abnormal swimming behaviour, loss of orientation, lack of surfacing activity, muscular spasms or lethargy) will be noted in the tadpoles especially at higher test concentrations, no adverse effect are to be expected at the lower levels. It can be assumed that cumulative signs of toxicity may be seen as the study progresses.

It is not the intention of the definitive test protocols to cause mortality or significant signs of toxicity. However, typically, the highest concentration tested in the metamorphosis assay will be near the maximum tolerated test concentration, or may be approximately one-third of the LC_{50}, depending on the dose response. As such, at the higher concentrations being tested there may be accumulation of toxicity noted during the exposure period which may result in adverse clinical signs (e.g. abnormal swimming behaviour, loss of orientation, lack of surfacing activity, muscular spasms or lethargy) being apparent (but not to the same degree as in the acute protocol) for a period of time resulting in cumulative toxicity/severity.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

21 day metamorphosis assay:

All adult animals which are subject to gonadotrophin injections will feel mild discomfort at the site of injection for a short period of time.

In the acute toxicity protocol it is anticipated that approximately 50% of the individuals will suffer mortality or adverse effects such as abnormal swimming behaviour, loss of orientation, lack of surfacing activity, muscular spasms or lethargy.

It is anticipated that approximately 15% of the individuals may suffer moderate effects such as abnormal swimming behaviour, loss of orientation, lack of surfacing activity or lethargy. These effects/clinical signs should not be experienced by the tadpoles to the same degree as in the acute toxicity protocol but due to the length of time that they may persist, cumulative severity may result in a moderate classification. Malformations may also be seen on occasion which will be assessed for severity.

Typically, the concentration range in the definitive test will be based on the results of acute toxicity tests or metamorphosis assays, and therefore it is not the intention to cause mortality. It is anticipated that no adverse effect will be observed at the lower levels.

All animals at some point will require moving to fresh test media, this is considered to cause mild stress in all animals. The discomfort will be transient in the majority of animals (>90%) and no additional action will be required.

Xenopus Eleutheroembryonic Thyroid Assay (XETA):

In the acute toxicity protocol it is anticipated that approximately 50% of the individuals will suffer mortality or mild to moderate effects such as abnormal swimming behaviour, loss of orientation or lethargy. Due to the low neurophysiological sensitivity of the life-stage of the tadpoles being used, death is classed as a moderate effect.

Typically, the concentration range will be based on the results of acute toxicity tests and therefore it is not the intention to cause mortality. It is anticipated that no adverse effect will be observed at the lower levels.

All animals at some point will require moving to fresh test media, this is considered to cause mild stress in all

animals. The discomfort will be transient in the majority of animals (>90%) and no additional action will be required.

What will happen to animals at the end of this project?

- Kept alive
- Killed

A retrospective assessment of these predicted harms will be due by 21 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Currently there are no validated non-animal alternatives to replace the whole animal toxicity tests that are mandatory for the evaluation of new and existing chemicals to assess their safety and efficacy. To assess morphological development due to effects on the thyroid axis, the use of a live animal is a necessity. Xenopus laevis is proposed as the test organism as it is known to be widely available, obtainable throughout the year and relatively easy to maintain. It is representative of the more sensitive species and a large literature base is available to provide background information.

Which non-animal alternatives did you consider for use in this project?

Currently there are no validated non-animal alternatives to replace the whole animal toxicity tests that are mandatory for the evaluation of new and existing chemicals to assess their safety and efficacy.

Why were they not suitable?

Currently there are no validated non-animal alternatives to replace the whole animal toxicity tests that are mandatory for the evaluation of new and existing chemicals to assess their safety and efficacy.

A retrospective assessment of replacement will be due by 21 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Amphibian metamorphosis Assay:

Numbers have been calculated based on running six LC_{50} tests and six definitive tests per year over the five year duration of this project licence.

Xenopus Embryonic Thyroid Assay

Numbers have been calculated based on running twelve LC_{50} tests and twelve definitive tests per year over the five year duration of this project licence.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Regulatory guidelines dictate numbers of organisms exposed per concentration and the spacing factor employed for definitive stage testing. Statisticians are consulted to ensure studies are designed to maximise the strength of replication in order to use the minimum number of organisms per vessel.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The use of a range finding phase of testing where existing information of the toxicity of a chemical is not available reduces the risk for repetition of the full metamorphosis or Xenopus Embryonic Thyroid Assays using high numbers of tadpoles or eleutheroembryos respectively, as a suitable range will be identified which aims to ensure the study endpoints are achievable.
Whenever possible, the maximum spacing factor will be employed when setting concentration ranges.
The priming and induction procedures utilised during the production and collection of eggs, in preference to relying on natural mating procedures, ensure that the minimum number of adult Xenopus laevis are used (e.g. 3 tanks of 3 pairs per spawning event), in order to achieve the maximum fecundity levels.
It is aimed that approximately 5000 eggs will be produced from the spawning event, from which the tadpoles from the spawn with the highest hatching success are selected and a minimum number (approximately 800) of these individuals are raised to the independently feeding stage.
A retrospective assessment of reduction will be due by 21 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Adult frogs used to produce eggs will suffer mild pain at the site of injection for a very small amount of time. As eggs are required for these protocols there are no other options available other than using adult animals.

Amphibians represent vertebrates and one of the highest trophic levels in the aquatic ecosystem and are of particular importance in the testing scheme because of their developmental life-history and sensitivity to

chemical substances, making them important ecological indicators. The species and stage of tadpole development employed are considered to represent the most suitable model to identify effects of exposure in the environment. The objectives and protocols are aimed at assessing the impact of a substance on the survival and development of Xenopus tadpoles, and therefore on the potential for further effects on the ecosystem, using mandatory tests that follow internationally accepted test designs. Due to the low neurophysiological sensitivity of the life-stage used during XETA, death is classed as a

moderate effect and as such this life stage (tadpole) will be the one that suffers the least during this type of testing.

Why can't you use animals that are less sentient?

As eggs are required for these protocols there are no other options available other than using adult animals. As the objectives are aimed at assessing the impact of a substance on the survival and development of Xenopus tadpoles, it is essential that living animals are used such that the development of these animals can be assessed. These animals are considered to be the least sentient species at this life stage used in this project licence where a meaningful assessment can be made on their development. The life stages that are required to be used are specified in the relevant OECD test guidelines.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Any refinements identified during the conduct of tests under this licence, as experience with these species is gained, will be assessed and put into common practice where possible. Methods for handling and identification have been designed within the field of amphibian laboratory use in order to minimise the chance of causing stress and pain in the adult Xenopus.
Appropriate handling of the adult organisms will ensure restraint procedures are effective for injection purposes and therefore ensure time taken for completion of injection procedures is minimised.
When necessary, any identification of the adult stock animals will take place using non-invasive methods e.g. photographic records in preference to toe clipping, tagging or microchip implant. Tank labels will be used to identify groups of animals.
The minimisation of excessive stress and rapid changes to environmental conditions, especially during movement, cleaning of vessels and manipulation of larvae, has been identified as a key factor in the optimisation of using Xenopus laevis. Husbandry and exposure conditions, and the associated techniques, will be well defined to ensure noise, vibration and activity levels within the laboratory are kept to a minimum. Environmental conditions e.g. temperature, pH, dissolved oxygen, light levels and water flow rates will be controlled and maintained.
Regulatory systems that require specific test requirements may allow little discretion in the species used. However, the most appropriate species, particularly in terms of species sensitivity and availability of background data, as well as the species having the lowest neurophysiological sensitivity, are chosen. Thus the most appropriate species is chosen on scientific grounds rather than custom and practice.
All scientific procedures using animals are performed in accordance with UK Good Laboratory Practice regulations. Standard Operating Procedures define animal welfare practices and experimental procedures. Licencees are fully trained and competent in the appropriate procedures and are familiar with the signs of pain, discomfort or distress in the species with which they are working. Training records are maintained to document training levels, retraining and competence. Staff are encouraged to identify and encourage improved methods particularly with regard to procedural methods, housing and handling of animals.
Animal tests are designed and conducted in every case so that any actual or potential pain, discomfort, or distress to the animals is minimised or alleviated by choosing the earliest endpoint that is consistent with the scientific objectives. The term "endpoint" is defined as the point at which an experimental animal's pain and/or distress is terminated, minimised or reduced, by taking actions such as killing the animal humanely or giving treatment to relieve pain and/or distress.
The ultimate purpose of the application of humane endpoints to ecotoxicology studies is to be able to accurately predict severe pain, severe distress, suffering, or impending death, before the animal experiences these effects. However, the science of ecotoxicology is not yet at the point where such accurate predictions can be made prior to the onset of severe pain and distress. It is possible at this time to identify pain, distress and suffering, very early after their onset by careful observations of animals on test. This test facility is fully committed to the implementation of the recognition, assessment and use of clinical signs as humane endpoints for test animals.
Before starting a safety evaluation study, consideration is given to relevant background data or information supplied with the test material, together with databases for similar chemicals or substances or previous

experience with other sponsor products. Thus, the type and severity of the abnormal signs of toxicity, particularly in terms of time of occurrence in relation to time of dosing may be anticipated. Technical and animal welfare staff will be alerted to what signs to look for.
Studies likely to cause significant acute adverse effects are scheduled to start as early in the working day as possible. This ensures that the critical period for the animals occurs during normal working hours when the frequency of observations can be maximised or increased depending on the potential for increasing pain or distress.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The proposed work will be conducted according to standardised test guidelines. These guidelines incorporate the scientific rationale for national regulatory requirements and are peer reviewed by scientific and industry experts to reflect current scientific knowledge and ethical standards in animal experimentation. Completed studies are peer reviewed by Competent Authorities, the company Registration Department and Sponsor representatives, and the comments received from post study peer reviews are used to further refine subsequent testing. Animal studies are not initiated until written confirmation is received from the sponsoring Company that they are needed to satisfy a regulatory authority and that funding to cover the full cost of the studies will be provided. The company Project Management Department (PMD) co-ordinates major notification projects and liaises with the Study Directors in charge of each study to ensure that unavoidable animal studies do not commence until the results of related non-animal studies are known.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

All staff involved in animal testing at maintain an up to date outlook on animal welfare considerations via the attendance of relevant meetings, review of publications in journals and other sources, and face to-face meetings with other individuals working in the area of ecotoxicology or aquatic husbandry. This ongoing knowledge building allows for any refinements that are identified to be introduced into standard procedures.
A retrospective assessment of refinement will be due by 21 January 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

49. Effects of neurotransmitters on metabotropic receptors in hippocampal neurons

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

neurobiology, receptors, ion channels

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The principle aim of this project is to understand how naturally occurring chemicals known as neurotransmitters exert their effects on the activity of brain cells, neurons, by binding to specific proteins on their surface that are called metabotropic receptors. In particular, we will be investigating the effects of neurotransmitters on adult mature and newly-generated granule neurons found in a key area of the brain, the hippocampus, which plays a central role in many brain functions including learning and memory.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Neurotransmitters play an important role in regulating the activity of neurons. These are released often in response to changes in behaviour. Certainly, alterations in their levels in the brain have been associated with many disorders including cognitive dysfunction and neuropsychiatric diseases such as anxiety and depression. It is, therefore, important to understand how these exert their effects on neurons.

We will be investigating the role of two particular neurotransmitters, acetylcholine and glutamate, acting on specific receptors on hippocampal adult mature granule neurons. These neurotransmitters play a key role in many brain functions such as learning and memory and medicines that bind to their specific receptors are currently in clinical development for the treatment of many disorders including dementia and neuropsychiatric disorders. Moreover, altered hippocampal granule neuron activity influences processes such as memory acquisition and consolidation and stress resilience. Despite this, we know very little about how acetylcholine and glutamate exert their effects on these neurons and the functional consequences of this. In this project, we will investigate this. The information generated will significantly advance our understanding of the mechanisms underpinning physiological processes such as learning and memory. It might also be beneficial in identifying new forms of treatment for disorders such as dementia and Alzheimer's disease.

The dentate gyrus is also one of two areas in the adult brain that can generate new neurons. These so called 'adult new-born neurons' have been suggested to significantly influence learning and memory during their development. Indeed, a reduction in the number of these neurons has been associated with cognitive deficits in Alzheimer's disease patients. The work proposed will substantially enhance our understanding of how acetylcholine and glutamate influence adult new-born neuron development and will be beneficial in designing new therapies for the treatment of disorders such as Alzheimer's disease.

What outputs do you think you will see at the end of this project?

We expect to generate novel information on how neurotransmitters regulate information processing in adult hippocampal neurons, which play a critical role in affecting normal processes such as learning and memory formation. In particular, the information will, for the first time, shed light on how two neurotransmitters, acetylcholine and glutamate, affect hippocampal neuronal activity when they activate receptors that are localised to a specialised part of neurons, the axon. This is important as digital, all or none signals known as action potentials are generated in axons. Action potentials play an essential role in determining a neuron's activity. Changes in hippocampal neuronal activity will affect our ability to learn as well as other forms of behaviour such as our levels of stress. Hence, this work will yield important information on the mechanisms underpinning these processes. It is anticipated that we will present this work at scientific meetings either in the form of posters or as PowerPoint presentations. Moreover, final datasets will be published in highly respected peer-reviewed neuroscience journals as we have previously done.

In addition to the above, the hippocampus is one of two brain regions where it has been determined that new neurons are generated. At present, we have very little knowledge about how neurotransmitters affect the development of these neurons. It is anticipated that this new knowledge will significantly advance our understanding of their development and will be of considerable interest to academic and industrial scientists. We will, thus, make every effort to widely disseminate this information by presenting complete datasets at scientific meetings and publishing final datasets in peer-reviewed journals that are fully open access.

Who or what will benefit from these outputs, and how?

In the short term, the findings will mostly advance our knowledge of how hippocampal neurons function and thus will be of immediate interest to academic scientists.
Since acetylcholine and glutamate metabotropic receptor stimulators are currently in clinical development for the treatment of multiple disorders including dementia, Alzheimer's disease and neuropsychiatric disorders, we anticipate that the information generated will be useful for those scientists working in industry who are interested in developing more targeted drugs with an improved side-effect profile for these disorders. This is expected to be a medium- long term impact of the findings.

How will you look to maximise the outputs of this work?

We already collaborate with many scientists in the UK, Europe and USA and will share datasets with them as appropriate as soon as we can.
Moreover, we will be presenting data at scientific meetings attended by academic and industrial scientists and medical staff, thereby ensuring that we will be disseminating the information as widely as possible.
We will publish all final datasets, regardless of whether the approaches are successful or not, thereby ensuring that the information is in the public domain.
Finally, we will take part in public engagement events such as the Royal Society Summer Scientific Fair to convey our findings to the public.

Species and numbers of animals expected to be used

- Mice: 6400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will be using juvenile and adult mice for our work. We have selected mice for our work as these breed well in house and mice with specific genetic modifications that would be particularly useful for addressing our question are readily available. Hence, the tissue from these animals is readily available for our experiments and will provide a reliable and consistent source for generating data.
We will be using adult mice as we are interested in understanding how neurotransmitters affect adult neuron function.

Typically, what will be done to an animal used in your project?

Typically in most experiments, mice with particular genetic modifications (transgenic mice) will be bred. The offspring will be genotyped to identify wildtype and genetically modified mice. When these mice are adult (5-8 weeks old), we will be terminally anaesthetise them and obtain brain sections from them to analyse the effects of neurotransmitters on particular metabotropic receptors in the hippocampus.
In a subset of experiments, particularly if transgenic mice are not available, we will surgically inject viral vectors that have been proven to be safe, into the hippocampus of adult mice to specifically modify the expression of particular proteins in the neurons that we wish to study. 2-3 weeks after this procedure, when the animals have recovered from this procedure and there is maximal expression of the viral vectors, we will terminally

anaesthetise the animals and obtain the brain sections to analyse the function of neurons when specific metabotropic receptors are activated.
In the event that transgenic mice or viral vectors for genetically modifying the expression of particular proteins are not available, then we will consider the use of chronically treating wildtype adult mice with particular pharmacological agents that modify the activity of particular proteins. If these are available and have been proven to be safe and harmless, then these agents will be continually administered via a device known as an osmotic mini-pump which is surgically implanted into mice. After a suitable period of treatment, animals will be terminally anaesthetised and brain sections obtained to study the effects of neurotransmitters on specific metabotropic neurons in adult mice.

What are the expected impacts and/or adverse effects for the animals during your project?

The transgenic mice that we wish to use are not expected to exhibit any abnormal behaviour or show signs of distress. These mice breed normally and develop normally too. Further, terminally anaesthetising mice to obtain the brain tissue is expected to cause minimal distress.
Mice will have to undergo surgery for the injection of injecting AAV or lentivirus viral vectors into specific areas of the hippocampus or implantation of osmotic mini-pumps for chronic pharmacological treatment (Protocol 2). Surgery is associated with several adverse effects including non-recovery from anaesthesia, wound infection and post-surgery pain. We, however, have adapted this procedure so that more than 95 % animals recover fully from anaesthesia and no animals suffer from either wound infection or post-surgery pain.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The breeding of transgenic mice and obtaining brain sections from mice is likely to cause minimal distress and is therefore likely to be at most of mild severity. This will apply to approximately 94 % of the animals that we will use.
We estimate that a very small proportion (6 %) will undergo surgery. This is categorised as moderate as it might result cause some temporary discomfort to animals.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Currently, we know very little about how neurotransmitters acting on metabotropic receptors exert their effects on neuron activity in real time. To study this, we need to obtain living, healthy brain tissue in which neuron function is intact. Thus, to understand how the function of individual neurons is affected by metabotropic receptors, it is essential to use animal tissue.

Which non-animal alternatives did you consider for use in this project?

Non-animal alternatives include
1) in silico advanced computer models.

2) human and stem cells that can be maintained in a dish.

3) human tissue.

Why were they not suitable?

In silico advance computer models: Whilst we have collaborated with scientists that are specialised in generating computer models, at present we have very little information available on how particular neurotransmitters acting on their specific metabotropic receptors affect neuron function. Thus, we cannot make these models or use existing models for this work.
Human and stem cells: Metabotropic receptors can be expressed in human cells that are maintained in a dish. However, human cells maintained in a dish do not express many of the naturally occurring signalling molecules that metabotropic receptors couple to when exerting their function. Indeed, our preliminary data show that there are significant discrepancies between how metabotropic receptors function in neurons present in living brain tissue and data obtained from human cells expressing metabotropic receptors. It is, therefore, important to use animal tissue to understand how native metabotropic receptors function and exert their effects in the brain. Stem cells can be used to generate neurons in a dish. However, this process is challenging and the properties of neurons derived from stem cells differ from those that are naturally occurring in the brain. For this reason, we cannot use stem cells for our work.

Human tissue: Postmortem human tissue is not suitable for our studies as we need to determine the effects of these neurotransmitters on living neurons in real time. The availability of healthy human living tissue obtained using surgical methods is limited and therefore, this method is not ideal for our work.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals have been estimated based on our experience and our data of the minimal numbers required to determine how neuron function is affected by neurotransmitters acting on specific receptors.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To estimate the animal numbers required for experiments involving surgical injection of viral vectors and implantation of osmotic mini-pumps for chronic pharmacological treatment, we performed power calculations using programmes such as G*Power. We estimated the number of neurons from which electrophysiological recordings or anatomical data would be required. Since we know that we can obtain at least one good electrophysiological recording from a neuron in slices obtained from one animal, we estimate that the number of neurons required to produce the required data is equivalent to the number of animals required.

Most experiments, though, involve breeding transgenic mice. These are heterozygotes to generate a mixture of wildtypes, genetically modified mice and heterozygotes. We use wildtypes and genetically modified mice for our experiments. However, we cannot determine the numbers of these generated by a given breeding pair. From our experience, we need to keep at least 4 breeding pairs per colony to allow generation of the required numbers of animals for all our experiments and for the colony to be maintained. Hence, the number of mice estimated for breeding is based on our current experience of the number of mice produced using 4 breeding pairs per mouse colony.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have optimised breeding by using animals between one month to 7 months for this purpose. The animals are housed in environmental enriched cages. This allows us to keep 4 breeding pairs at any one time and produces the required animals to complete our experiments.
Animals used for experiments are also group housed in enriched environments. This allows animals to be relatively relaxed, thereby ensuring that we get good quality brain tissue from them and obtain the maximum amount of data from each animal.

A small number of animals will undergo surgery. We have optimised the conditions required for surgery to ensure that more than 95 % animals undergoing this procedure have little post-operative pain or distress. Moreover, wherever possible, animals are group housed in environmentally enriched cages. Further, the viral vectors that will be used are those that have been shown to be safe. The genetic modifications induced by these vectors are not predicted to cause any harm.
For vectors and pharmacological agents that we have not used previously and for which there is no publicly available data, we will carry out pilot experiments to optimise these experiments.
We often share our data and materials with other scientists, particularly those involved in computational modelling, thereby enabling maximal use of the data that we generate.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will be using genetically modified mouse models for the majority of our work. These mouse models are commercially available. The genetic modifications have been shown to have little impact on breeding and the animals have a normal life-span and are able to eat and drink normally. Mice will be identified using genotyping and may be administered substances to induce gene expression.
For some experiments, we will be performing surgery to administer substances to alter gene expression or to manipulate receptor activity. The substances that will be used are those that have been proven to be safe to animals and humans and not predicted to cause distress or lasting harm to animals. Surgery itself is associated with pain and distress. To minimise this, we have optimised this procedure by using appropriate anaesthesia, aseptic techniques, monitoring body temperature and breathing, administering peri-operative analgesics and treating wounds with iodine to reduce infection. We find that using these conditions, animals are not distressed.
To quantify the effects of genetic modifications or changes in receptor activity, we will obtain brain tissue from animals. To obtain good quality tissue, we will terminally anaesthetise animals before embarking on this procedure. This will ensure that the animals experience little distress during this process.

Why can’t you use animals that are less sentient?

We will be investigating how neurotransmitters exert their effects on adult neurons as there is very little information available on this. For this, we need to use adult animals and obtain brain tissue from them that contains the neurons that we are interested. For most experiments, we will terminally anaesthetise animals to obtain the brain tissue required.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Biological Services unit staff and/or research staff monitor animals housed in the Biological Service unit at least three times a week. If animals are observed to be experiencing distress, we will monitor these more regularly at least twice a day and consult the NACWO and/or NVS on best practice.
For 5 days post-surgery, animals will be regularly monitored twice a day for signs of distress. Moreover, they will be weighed once daily to ensure that they are feeding and developing normally. They will be group housed in environmentally enriched cages.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will following ARRIVE guidelines which have been published and are available on the NC3Rs website.
In addition, we will following the published AWERB guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will attend seminars run by NC3Rs which are run by an NC3Rs representatives. Moreover, we will regularly visit the NC3Rs website a to be informed of these matters. Further, we are in regular contact with the NACWO and NVS who will also provide advice on how to implement advances in 3Rs during the during of the project.

Home Office

NON-TECHNICAL SUMMARY

50. Efficacy evaluation of products to support the health and welfare of farmed fish

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b) **Key words**

aquaculture, product development, vaccine efficacy, therapeutic efficacy, feed additives

Animal types	Life stages
Atlantic salmon (Salmo salar)	juvenile, embryo, neonate, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to evaluate the efficacy of products, including veterinary vaccines, therapeutics, feed materials, feed additives and biocides, intended for use in the production of farmed fish. The project will provide a service to commercial clients and research providers seeking to develop products for fish. The principal objectives are to provide data on the effectiveness of the products in relation to prevention and treatment of infectious disease and other pathologies, and impacts on performance and welfare.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

These studies are necessary to enable assessments of the effectiveness of the products for use in farmed fish, including regulatory assessment for the purposes of product licensing, to identify the preferred treatment procedure and to understand the modes of action of the products so that the products can be used safely and effectively.

What outputs do you think you will see at the end of this project?

The expected benefits of this project will be to:

1. Establish a platform which clients can access to develop products for aquaculture. A shortage of capacity at present is restricting the availability of new products for farmed fish.

2. Support the evaluation, development and licensing of new products to improve the health and welfare and performance of farmed fish. These products will be used by salmon farmers to reduce losses due to salmon lice and other diseases and to improve production efficiency.

3. Generate high quality data to ensure that new licensed products are demonstrably effective. These data will be produced to the internationally recognised quality standards required by relevant regulatory authorities.

4. Support the production of fish as food that is safe, healthy and nutritious, economically sustainable, environmentally acceptable and produced to the highest animal welfare standards.

5. Tackle biological challenges which threaten the sustainability of an industry which supports jobs and economic activity in remote areas.

Who or what will benefit from these outputs, and how?

The benefits will be realised by:

1. Farmed fish which will benefit from improvements in health and welfare.

2. Husbandry and veterinary staff who will benefit from access to new tools to maintain and improve the health and welfare of animals in their care.

3. Aquaculture producers, processing companies and retailers who can expect marketing and price advantages based on reduced losses and more efficient production of fish with higher health and welfare standards.

4. Supply chain companies who will benefit from opportunities to develop new products and services.

5. The consumer who will benefit from access to food produced using products which have been developed according to established and assured safety and welfare standards.

How will you look to maximise the outputs of this work?

We will advertise our capabilities and expertise within our target market so that a wide range of clients can take advantage of these.
We will encourage our sponsors to publish study findings, including negative findings, where appropriate.
We will offer experimental models to research groups for testing novel products. Knowledge of these models will be shared and may be published.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The fish species and life stages used in this project are representative of the farmed fish species and life stages for which the products are being developed.
The developers of the products and Licensing Authorities responsible for approval of new products require data from these 'target' species for decision-making and formal regulatory assessment.

Typically, what will be done to an animal used in your project?

A test product may be administered to experimental fish, for example by voluntary feeding daily for up to 12 weeks or by immersion or by intra-peritoneal or intra-muscular injection under temporary anaesthesia, at a dose which has previously been shown to be safe.
In studies to determine the efficacy of the product against pathogen challenge, fish may be challenged with a pathogen under controlled conditions. Pathogen challenge may be before or after administration of a test product. Fish will be held in tanks and may be anaesthetised or euthanased and sampled during the study. Samples will typically collected in order to assess infection status, for example to count the number of attached sea lice at a certain time point.
In fish performance studies, fish will be held in tanks and may be anaesthetised or euthanased and sampled in

order to evaluate key performance indicators including growth and feed conversion rate, nutrient retention and physiological function.

What are the expected impacts and/or adverse effects for the animals during your project?

The expected impacts are those associated with disturbance and handling of animals for dosing and controlled infection with the pathogen of interest. Short term effects are expected to include increased ventilation rate, skin darkening and inappetence with full recovery within 24 h.
In the case of sea lice challenge and sea lice product efficacy studies, sea lice infestation may cause irritation leading to increased jumping behaviour and inflammation of the skin in approximately 20% of fish which will resolve within a few days. The other 80% of fish are expected to experience no adverse effects.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of fish in sea lice challenge and sea lice product efficacy studies will experience Mild severity.
Approximately 20% of fish in each study are expected to experience Moderate severity.
All animals used in fish performance studies are expected to experience Mild severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The project will test the effectiveness of products administered to the intended target species which will be a species of farmed fish. Due to the complex nature of the interaction between a product and the animal (and pathogen where used) the effectiveness of the products cannot be evaluated adequately using non-animal alternatives.

In studies conducted for regulatory purposes, the use of relevant animal models is a requirement of the regulatory authorities in order to properly assess the product.

Which non-animal alternatives did you consider for use in this project?

Computer simulation models. *In vitro* bioassays.

Why were they not suitable?

These non-animal models are either not available or not well-enough developed to provide the required high level of confidence in the results.
In vitro bioassays will be used for initial product screening.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For each type of study, fish numbers are based on the typical study design requirements (based on regulatory guidelines) and on published data and/or past experience of appropriate sample sizes.
The estimated total number of animals is based on expected demand and capacity for approximately 32 studies using standard study designs.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The study designs use prior data to estimate the likely magnitude of variation in response due to random effects and the level of treatment effect which is practically valuable. The discrimination of the study design (i.e. its ability to distinguish treatment effects from random variation) is maximised by minimising the effects of random variation by the practice of using fish of similar age, source, size range and history, similar experimental tanks and consistent environmental conditions across each study.
When designing new challenge models, guidance on study design will be sought from a biostatistician. Study designs for indivdual experiments will be evaluated by a biostatistician as part of the experimental and ethical review procedure.

Group sizes for voluntary feeding studies are restricted by the requirement for fish to show a uniform feeding response. A minimum group size of 20 fish is used in these studies since this is considered to be the minimum necessary to overcome social hierarchy effects and provide an acceptable feeding response in the majority of individuals in the population.
Smaller numbers of fish may be used where dosing is by immersion, oral gavage, injection or topical administration.
In time series studies, for example to determine the duration of efficacy, where a group of fish may be treated with an experimental product and sub-sets challenged and sampled at intervals, numbers of fish reflect the number of sample points and the number of individuals required for sampling at each point. In studies which are required to generate quantitative data, numbers of fish used at each point are those necessary to provide an accurate and precise measure of the magnitude of response. This is determined separately for each study using sample size calculation methodology but is typically in the order of 10-20 fish per time point.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies may be used to determine the magnitude of effect of treatment and thereby the number of animals/samples necessary in pivotal regulatory studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques

during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The sea lice challenge procedure uses the number of fish necessary to support the required numbers of parasites without over-infestation of fish and without the use of too many fish. Individual animals may be used for a series of challenges when the harm caused by the additional challenge is less than the harm caused by acclimatising, challenging and habituating new fish to attachment of the parasites. The experimental challenge procedure is well established and refined so that settlement rates are predictable.
A sea lice product efficacy test aims to minimise random variation (noise) in sea lice data so that it is possible to detect effects due to the test product, statistically, with use of the minimum number of fish.
A fish performance study aims to evaluate the performance of fish receiving the product under practical conditions of use. High welfare standards are necessary to achieve commercially realistic growth and feed conversion rates and experimental procedures are minimal in order to limit any risk to welfare and reduction in performance.
Products used in these studies will be used at dose rates or inclusion levels which have been shown elsewhere to be within the safe range.

Why can’t you use animals that are less sentient?

The species and life stages used are those for which the products are being developed. Reliable data is important to ensure the safety of farmed fish and the consumer. Less sentient animals have not been shown to provide data which can be reliably extrapolated to the target animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Frequent monitoring for adverse effects using established criteria as described in the relevant protocols. Refinement of the procedure used for identifying, describing and recording clinical signs, for example identification and specific focus on relevant new operational welfare indicators identified in future publications, increased use of video monitoring to avoid disturbing fish and refinement of terminology used to describe clinical signs.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

NC3Rs PREPARE and ARRIVE guidelines
Festing, M.F.W., Overend, P., Borja, M.C. and Berdoy, M. (2016). The design of animal experiments. 2nd Edition. Laboratory Animals Handbook No. 14.
Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare. 351 pp
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How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

NC3Rs Newsletter and Website.
Norecopa fish as research animals website
Local 3Rs Group.

Relevant training courses.
Communication with sponsors and colleagues working in the field.

Home Office

NON-TECHNICAL SUMMARY

51. Embryogenesis, stem cells and cell fate decisions

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how cells choose and maintain specific fates during development of the mammalian embryo and in the adult animal, how this leads to disease when the processes go wrong.
A retrospective assessment of these aims will be due by 22 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

During the development of an animal, cells have to undergo decisions of cell fate, choosing which path to follow. Similar decisions are made throughout life by stem cells and progenitors in many tissues. These decisions rely on intrinsic factors, such as transcription factors, and extrinsic signals, which together establish gene regulatory networks that define specific cell states. These have to be coordinated in time and space to generate functional tissues, organs and the animal, where little in the latter is static: cells are constantly having to be replaced due to normal wear and tear and to cope with changing physiological states, trauma, and disease. The main purpose of the work to be conducted under this Project Licence is to provide fundamental knowledge on cell fate decisions in specific biological systems, notably the gonads, CNS, pituitary, gut, and sensory systems, which is relevant to how these develop, mature, and age normally. However, there are many situations where decisions of cell fate are aberrant or discordant. This can lead to infertility or embryo failure, to congenital disorders, disorders affecting maturation, health span or aging, or to cancer. There can also be aberrant responses to environmental factors. Improved understanding of underlying mechanisms can lead to improved diagnosis and/or novel forms of treatment.

What outputs do you think you will see at the end of this project?

1. Improved understanding of gene regulatory networks in the early gonad will not only inform clinical cases of Disorders of Sex Differentiation (DSDs), but be of broad significance in studies of organ formation.

2. Improved understanding of gene regulation during gonadal development will also inform clinical cases of Disorders of Sex Differentiation (DSDs), as it is become apparent that many are due to regulatory mutations. We also anticipate that this work will provide general insights into both temporal and spatial control of gene activity, especially for genes such as *Sox9* which are active and have critical roles in many tissues.

3. Improved understanding of gonadal sex maintenance and reversal will lead to new insights into cellfate reprogramming and organogenesis, both of which could be of potential importance for regenerative medicine. More directly, this could be of clinical benefit for some cases of DSDs, giving new options for treating patients, such as in cases where ovotestes are present it may be possible to turn the whole gonad into a testis or ovary, or to convert the entire gonad, either to match chromosomal sex or, perhaps, gender identity.

4. Improved understanding of female reproductive function, fertility and premature ovarian failure, could help inform new strategies to manage or maintain fertility in women.

5. Developing methods to obtain gonadogenesis and gametogenesis in vitro, could provide information relevant to DSDs and to causes and potential treatments for infertility. It is very difficult to study the etiology of DSDs given that the phenotypes develop in the embryo in utero, and they are generally recognised at birth or, often, at puberty. The only alternative at present is to make and study an animal model, which, while useful, may not always accurately reflect the human situation. (N.B. Our work deriving Sertoli-like cells in vitro from pluripotent stem cells has already contributed to one study (yet to be published), where patient derived cells were unable to give rise to Sertoli cells, unlike controls, showing that the defect was in primary sex determination). Current efforts to derive sperm or eggs in vitro from pluripotent stem cells reveal that co-culture with somatic cell types from the gonad is essential for primordial germ cell-like cells to progress into later stages of spermatogenesis or oogenesis. Our culture systems, if validated in animal studies, will help these endeavours, which are important both to allow detailed study of human germ cell development and, in the long term, to provide ways to treat infertility or even as a route to correcting deleterious gene variants in subsequent generations.

6. The use of animal models to provide improved understanding of the mechanisms underlying sex bias in human diseases will be of clinical benefit in terms of improved diagnosis and perhaps options for treatments. Our current work on Hirschsprung's Disease provides an example, where two mechanisms that could contribute to the distinct sex bias have been identified. Work on sex differences in the biology of neural stem cells is of potential relevance to understanding and perhaps eventually treatment of a range of CNS disorders that affect one sex more than the other, such as depression.

7. In addition to providing novel fundamental insights, our studies on intrinsic versus extrinsic control of CNS development and disease could be of benefit in providing new options for avoiding or treating diseases that have previously thought to have their origins within the CNS.

8. Our work on the role of SoxB1 and SoxE genes in development, from the early embryo to neural stem cells in the adult, has already led both to new fundamental knowledge and to clinically relevant findings. These range from Sox2 being recognised as a gene essential for pluripotency and hence to the development of iPS cells, to studies on the decline of neural stem cell populations in ageing. We expect our further studies on these genes to continue to give new knowledge.

9. Improved understanding of neural stem cell niches will contribute to basic knowledge, but also potentially to the development of new clinical options for treatments of CNS defects and trauma. Neural stem cells have already been used in attempts to treat a range of diseases as well as conditions such as stroke, but with limited effectiveness. Providing other niche components, whether factors produced by cells or the cells themselves, may be beneficial.

10. Gliomas and glioblastomas have proved to be very hard to treat. Our work on this topic is designed to reveal more about the origins of these aggressive tumours, and the knowledge gained will potentially provide new ideas for treatment options.

11. Our work on the pituitary and hypothalamus, and stem and progenitor cells in both, has already led to new insights. Ultimately, we hope this work will lead to better, more physiological options to treat a range of clinical disorders that involve deficiencies in pituitary hormones and/or in hypothalamic function. These can be congenital or occur after trauma, disease (including cancer), or may be due to current treatment regimes.

12. Pituitary tumours are relatively common, but not well understood. Our work may provide new treatment options, especially as surgery on the organ, which is centrally located below the brain and highly vascularised, is often very challenging. For example, via the use of drugs to disrupt specific cell signalling molecules that our current work suggests are required for tumour growth or that might reduce *Sox2* expression, which is also associated with tumour growth.

13. Our work on the development and function of endocrine organs and sensory systems is mostly to provide fundamental knowledge of the role of specific genes. However, this knowledge will be relevant to diagnosis and management of patients and may eventually provide new treatment options.

We will publish all of our findings in open access journals, with data in a reusable form. Moreover, any genetically altered mice produced as part of this PPL, which should be of benefit to other researchers, will be made freely available, as we have done so in the past.

Who or what will benefit from these outputs, and how?

There are likely to be multiple beneficiaries from the outputs above. These will first include other scientists and clinicians involved in similar studies or who are interested in the systems we are exploring. Benefits to patients may come within a few years, perhaps some within the time frame of this PPL, from improved diagnosis and from better management of disorders or trauma. In the longer term, perhaps in 5 to 15 years, we would expect our work to lead to new treatment options.

How will you look to maximise the outputs of this work?

Alongside the publication of primary research papers in open access journals, we will present our work at meetings, ranging from small focussed workshops to large international conferences. Critically, we will communicate negative results and approaches as well as those that are positive.

We collaborate widely, both internally within the host establishment, and externally with scientists and clinicians based in the UK and in several other countries (including currently France, Spain, Israel, the USA and Canada, and Australia). We share details of approaches and data generated with our collaborators, which allows for improved development of methods and better synthesis of findings.

Where we develop new transformative methods, we will publish the protocols as papers and post them on bioRxiv.

Species and numbers of animals expected to be used

- Mice: 95,700

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use mice for all the projects covered in this PPL. Mice are chosen because of the powerful techniques and knowledge available for this species in terms of genetics, cell biology, embryology, physiology, reproductive biology, and behaviour, but also as they have relevance to the human situation.

Evolutionary comparisons can be very informative. A separate PPL will cover our work with the chick. In addition, our mouse work will include a small number of chimeric animals that will be generated by admixing mouse embryos with cells (such as embryonic stem cells) from another mouse or small mammal, such as the rat. We will use this strategy to replace the elements of, for example, the developing brain and spinal cord so we can observe some aspects of how these systems develop, and how much is determined by the cells of the system and how much by the surrounding tissues. This approach can be used to study aspects of physiology or disease that affect other mammals where it would be difficult to conduct the experiments in these mammals. This could include humans, although this is not part of our current programme of work.

With respect to life stages, our work ranges from preimplantation embryos all the way to ageing adults. This range reflects, in part, the focus of the lab on certain genes, for example *Sox2*, that function in cell fate decisions throughout many or all these stages, but also the importance of understanding the changes that take place during organ development and maturation and altered physiological circumstances, including ageing.

Typically, what will be done to an animal used in your project?

Because we often use genetic approaches, much of our work involves breeding, including GA animals, and harvesting embryos or tissues from postnatal animals after they have been killed (using a schedule 1 procedure or fixation/perfusion) for detailed analysis of phenotypes. We will use hormone injections to control aspects of reproduction, e.g. superovulation when making genetically altered animals. Quite often we make use of substances, such as tamoxifen, to induce a genetic alteration, e.g. a conditional loss- or gain-of-function of a specific gene, as well as to follow cell fates or isolate specific cell types (e.g. by activation of a fluorescent reporter gene).

For a smaller number of animals, we will use some surgical techniques, always employing appropriate anaesthesia and analgesia, and in consultation with the NVS. These include methods associated with the production of genetically altered mice, including chimeras, and studies on reproductive biology, such as embryo transfer, vasectomy, ovariectomy and orchidectomy. Some of these methods are also used for routine maintenance or preservation, as frozen embryos or sperm, of specific genetically altered mouse lines. We also use surgery in a small number of animals to explore the consequences of removing target organs (such as the adrenal gland) of the pituitary on the hypothalamic-pituitary axis.
Our work has shown that this leads to activation and differentiation of stem cells within the pituitary. Occasionally more than one organ will be removed (e.g. adrenals and testes) for the same reason, and to explore if the

effects are additive or affect distinct cell populations. As part of this PPL, due to having derived a new genetically altered mouse line that permits cell fate mapping without the use of tamoxifen (which disturbs reproductive function and the hypothalamic-pituitary axis) we will investigate the effects of changing physiology, such as puberty, pregnancy, lactation, on the pituitary stem cells. This will not necessarily involve surgery, but may involve additional injection of relevant substances including into pregnant mice, where the main object is to study the mothers rather than the embryos.

We will also use substances, such as EdU or BrdU, to look at cell proliferation in tissues after harvesting. These substances are introduced by injection or gavage, into pregnant females or live-born animals, and may be carried out multiple times over a few days, followed by a variable period prior to the animal being killed and the tissues analysed. This can be to follow cell fates over this period, or to examine the consequences of an induced mutation at different life stages, or to carry out specific assays, such as a ‘label-retaining’ assay, often used to identify quiescent stem cells.

Some projects, involving few animals, make use of conditional or inducible genetic systems or drugs to deliberately kill specific cell types. This can be to study the consequences of their loss on the organ system under study, such as the pituitary or stem cell niches in the brain. And a new project will explore the effects of radiation or anti-mitotic chemicals on the hypothalamic-pituitary axis, which is known to be compromised after radiotherapy or chemotherapy. Most of these experiments are short term, lasting days to a few weeks, before the consequences are analysed. Alternatively, these methods may be for studies using blastocyst complementation, when specific cells, such as neurectoderm progenitors, are deleted in the developing host embryo, but replaced by cells differentiating from pluripotent stem cells introduced at blastocyst stages. In this case, the effects are assessed in a stepwise manner, looking at embryos less than two thirds through gestation, then at embryos shortly before term, before allowing any chimeras to be born. If these are viable, the animals will be kept longer for subsequent phenotype analysis, but with careful monitoring.

For some projects, but again involving small numbers of mice, we need to introduce substances and/or cells into the brain, which will also involve surgical procedures, including implantation of canulae and osmotic minipumps, injection needles, and, electrophysiology. Substances and/or cells may also be introduced into the developing brains of embryos in utero, which also requires procedures to be performed on the pregnant female. Imaging methods, such as ultrasound, may be used to guide positioning of needles. For monitoring changes in physiology or hormone levels, we may implant a cannula into a blood vessel to sample blood.

A small number of control and genetically altered mice, some after surgery, may be subject to (mild) learning and memory or other behavioural tests.

Some projects are also concerned with cancer and/or ageing. In the majority of cases, those mice expected to develop tumours will do so as a result of a particular breeding protocol involving certain genetically altered strains, such as with null or conditional mutations in *p27*. In other situations, the tumours will develop through injection of cells that are known to lead to tumours, either from an original tumour, such as a gliobastoma, or from pluripotent stem cells that can give rise to teratomas or teratocarcinomas. In all these cases we monitor tumour development, using imaging methods where possible, as detailed in the relevant protocol. Extensive

post-mortem tissue analysis will be performed to maximise the information obtained from each animal. For certain types of tumours, such as those developing in the pituitary due to mutations in *p27*, these generally only become apparent in older animals. The effects of ageing on stem and progenitor cells in the CNS and pituitary, and on gonadal sex reversal, also require some animals to be kept for more than a year.

What are the expected impacts and/or adverse effects for the animals during your project?

For the majority of our experiments, wild type and genetically altered liveborn mice should experience no more than mild effects. For strains that carry harmful mutations, the lines are maintained as heterozygotes, which themselves tend to have mild or no apparent phenotypes. However, animals may be crossed to generate homozygotes or compound mutations, where stronger phenotypes occur, including embryonic or postnatal lethality, or reduced lifespan. It will be necessary to study embryonic stages and to keep some animals with harmful mutations until the phenotypes develop, in order to study how they arise. We will kill animals before end points for the relevant severity band are reached.

The types of phenotype range, according to the specific gene being altered and the type of alteration, from complete or partial sex reversal and/or infertility, craniofacial defects, loss of hearing or vision, CNS defects, abnormal behaviour and/or defects in learning and memory, epilepsy (although this can be managed by careful handling), hypopituitarism, tumours, to shortened healthspan or lifespan. With mutations affecting some genes, there may be phenotypes outside the tissues we study, which can lead to lethality. An example would be kidney defects in addition to sex reversal with mutations in *Wt1* or in addition to enteric nervous system defects with mutations in *Ret*. In these cases, the animals are killed prior to the kidney defects becoming deleterious shortly after birth. In other cases, we are interested in the origins of a deleterious phenotype, such as the failure of the enteric nervous system to colonise the distal gut in mouse models of Hirschsprung's disease, and not the problems this leads to. We therefore kill any newborn animals with the relevant genotype before symptoms of the disease, such as 'megacolon', become evident.

The frequency, type and severity of any adverse event depends on the procedures being used, together sometimes with genetic status, including the genetic background of the strain. We endeavour to minimise the chances of these occurring, but the cause is sometimes unknown, such as the occasional death after administering tamoxifen. Depending on its severity, the duration of an impact or adverse effect may range from a small number of days to a small number of weeks, or even for much longer if fertility or behaviour are affected, with the animal being killed prior to it reaching the relevant end points as defined in the protocols.

For animals undergoing surgical procedures, most should only experience transient discomfort with pain being managed by anaesthesia and analgesia. They would be expected to recover fully within a few days. For some, the outcome may be more severe, notably after adrenalectomies there can be significant weight loss associated with 'salt wasting'. This can be managed by adding salt to their drinking water, but this fails to rescue the mice after 4-8 weeks. We therefore kill all such mice at a maximum of 3 weeks post adrenalectomy. If combined with tamoxifen treatment, animals that have been subject to adrenalectomy have a significantly increased risk of death about three days afterwards, which can be up to 50% (with no clear reason). For this reason, animals treated under this combined protocol (No. 11), which is used for a small number of animals, are maintained for no more than 7 days, and the protocol is classified as severe.

With aged animals, the chance of an adverse event, including unexplained death, is also increased. Such animals are therefore also maintained under a severe protocol (No. 9), although the majority do not exhibit severe effects.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severities for the mouse experiments are:
Mild; about 75% of the animals.
Moderate; about 23% of the animals.
Severe; less than 2% of the animals

What will happen to animals at the end of this project?

- Used in other projects

A retrospective assessment of these predicted harms will be due by 22 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our work is very much embedded in mouse genetics, and this requires breeding animals. Furthermore, most, if not all cell fate decisions in the embryo and adult animal take place within a complex environment, where events intrinsic to the cells are influenced by a variety of extrinsic signals, whether these are from neighbouring cells, involve molecules, such as growth factors, cytokines and hormones (which can act over considerable distances), are commensal with the animal, such as gut microbiota, or are part of the environment, i.e. are external to the organism. Moreover, most tissues develop in a complex way in three dimensions over time in a carefully orchestrated manner. Therefore, although some aspects of certain cell fate decisions can be studied *in vitro*, and we both use and develop such approaches, it is generally essential to study them *in vivo* (as a minimum to judge the suitability of *in vitro* systems to give meaningful information). This is best illustrated with a few examples relating to our work:

(i). Several distinct cell lineages give rise to the developing gonads and their continued interactions are required for appropriate gene activity leading to the development of either testes or ovaries. For example, in the mouse, the supporting cells arise from the coelomic epithelium overlying the genital ridge, the steroidogenic cells arise from mesonephric mesenchyme at early stages, and the germ cells are specified amongst extraembryonic mesoderm in the base of the allantois during gastrulation and then migrate back into the embryo and eventually to the gonad. Some connective tissue cells arise from unspecified mesenchyme in the early gonad, whereas endothelial cells that are critical to establish an arterial blood flow in the testis migrate from the mesonephros after Sry activity has triggered Sertoli cell differentiation. Early Sertoli cells also influence germ cells to enter mitotic arrest, and actively prevent them from early entry into meiosis, which is typical of the ovary. It is currently not possible to mimic all of these cell-cell interactions using cells maintained *in vitro*. We can culture the intact early gonad for periods of two to three days, which does allow us to follow some events in real time (and reduce animal numbers), but this still requires breeding to produce the animals.
(ii). To study postnatal gonadal sex reversal, such as when *Foxl2* is conditionally deleted, cannot meaningfully be studied in any in current *in vitro* system. While it is possible to culture isolated granulosa cells for a limited time, they tend to lose expression of critical genes and their normal phenotype. The same is true of Sertoli cells. Without a robust and reliable culture system that could maintain both of these cellular phenotypes, it would be impossible to address the consequences of deleting *Foxl2* in meaningful way. Moreover, it would not be possible to investigate how other testicular cell types differentiate, nor the morphological changes that accompany the changes from ovary to testicular-like structures.
(iii). The pituitary develops through a complex series of reciprocal inductive events between the oral ectoderm and the overlying ventral diencephalon. Some progress appeared to have been made several year ago to mimic aspects of this *in vitro*, beginning with ES or iPS cells; however, the structures reported failed to reflect the cellular organisation of the pituitary, or appropriate production of hormones. Moreover, these experiments have proved difficult to replicate. We can also grow stem cells from the pituitary for a limited time *in vitro* and, by changing conditions they can differentiate into each of the hormone producing cell types typical of the anterior lobe and we use this *in vitro* model to ask some questions about factors influencing stem cell self-renewal and differentiation. However, it has not been possible to reintroduce these to the pituitary *in vivo* to test if they retain relevant functional properties. Nor are these ‘pituispheres’ likely to have sufficient complexity to model the real organ or to be useful to address issues of the interactions of the pituitary with the hypothalamus and its target organs, which requires whole animal studies.
Over the last few years we have determined that the pituitary stem cells respond to changing physiological conditions. For example, the stem cell population is mobilised by estrogen treatment of males or by gonadectomy or adrenalectomy, but it is not known how a systemic signal affects the stem cells, whether this is via other cell types in the pituitary, or the hypothalamus, etc. We have also recently discovered that the stem cell population is itself complex. To answer these questions requires *in vivo* experiments. Moreover, as we move to explore how the stem cells respond to normal life events such as puberty, pregnancy, lactation, etc, these again have to be carried out *in vivo*.
(iv). To explore the role of specific *Sox* genes in CNS development and their association with the origin of specific tumour types or with ageing, similarly cannot be adequately replicated *in vitro*. For example, there is no *in vitro* model of hippocampus development. Addressing the consequences of abnormal CNS development on behaviour or learning and memory also requires *in vivo* experiments.
(v). There is now increasing evidence that many aspects of anatomy, physiology, behaviour, pathologies, and responses to treatment, differ between the sexes; and even when these appear similar, the underlying mechanisms may be different. These differences are likely to be due to direct effects of X and Y linked genes, to sex hormones made by ovaries or testes, or both. Moreover, these effects can be organisational (i.e. they are established during development, perhaps prior to any obvious difference), or activational (require constant input). Experiments to understand the mechanisms involved, the importance of which have been widely recognised in recent years, cannot be conducted *in vitro*.

Which non-animal alternatives did you consider for use in this project?

We complement our *in vivo* analyses with tissue culture models and organoids. These include the pituispheres mentioned above, as well as neurospheres and NS (neural stem) cell cultures for the CNS. We also make use of cell types obtained from mouse or human pluripotent stem cells (ES or iPS cells) via processes of directed differentiation *in vitro*. These can give rise to cell types typical of the CNS and, from our work, to the early gonad. We have also, with collaborators, attempted to use tissue engineering to construct 3-D models of neural stem cell niches, and plan to use similar methods to assemble gonad-like structures.

Why were they not suitable?

We can gain a certain amount of information from these *in vitro* systems, but there are serious limitations. It is not currently possible to replicate the complexity of mammalian tissue structures in culture models. In addition, while some molecular assays (e.g. RNAseq), suggest that the various cell types we have derived from pluripotent stem cells are similar to the endogenous cell types we wish to study, they are not identical. Moreover, the proof that we can derive and propagate relevant cell types, will depend on their ability to function when reintroduced into the relevant organ *in vivo*, which can be challenging and still requires animals. Finally, such cell or organoid cultures cannot permit research on aspects of biology such as reproduction, brain function, or physiology.

A retrospective assessment of replacement will be due by 22 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This estimate is based on several factors. It is based on our past experience, particularly over the past 5 years. We have also taken into account the number of researchers within the group who perform mouse experiments (currently 10, and it is likely to remain around this number over the next five years, with MSc students usually boosting this (currently two) for 3 to 6 months during each spring/early summer). We also continually re-evaluate the numbers of mice required for each experiment using power calculations. For this we access help from an in-house statistician when necessary. This will allow us to determine the number of animals required per experiment to give statistically valid results. Numbers of mice used for breeding are based on best practice, experience with each strain or combination of strains, and factoring in the likely proportion of the desired genotypes, including controls.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

When designing specific experiments within the overall project we estimate the minimum number of animals required to give robust answers. Most often this can be based on our prior experience or on published data. Often it is not necessary to use statistics (e.g. three transgenic lines giving the same pattern of expression

shows that this is correct), however, we perform statistical analysis whenever necessary. Where experiments involve physiological manipulation, or result in phenotypic and/or physiological consequences, for which we have little or no prior information, usually around 5 or 6 animals per treatment group (which will include sex as a variable when relevant and possible) are sufficient to obtain robust results. The design of quantitative experiments generally follows the ARRIVE guidelines and sample sizes may be set using power analysis. Any exceptions are where there is a degree of variability beyond our control (for example, where minor fluctuations in conditions together with threshold effects require more animals to be examined in order to have statistically significant results). We generally use a significance level of 5% and a power of 80%, estimating standard deviation from pilot experiments. We include advice taken from local statisticians as well as make use of online tools, such as the NC3Rs' Experimental Design Assistant.
For some important questions that we wish to address there can be a choice between using a mild procedure but many animals because the measurable effect is weak, or a moderately severe procedure with few animals because the effect is robust. Our choice will depend on the specific question and available resources, but it will most often be to use fewer animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We try to keep as few mice as possible by careful monitoring our mouse colony and good practice. Whenever possible and when there are no harmful phenotypes (or infertility) we maintain genetically altered mouse lines as homozygotes to reduce the numbers of animals required and to reduce the need to genotype. These may be crossed to another genetically altered strain or to wild type mice prior to beginning an experiment followed by intercrossing the heterozygote offspring when wild type and/or heterozygous animals are required as controls for the homozygotes. We also make use of fluorescence reporters that can also (in some circumstances) avoid the need for biopsies, especially for genotyping. To minimise breeding, lines under sporadic use are maintained at lower levels. We also use cryopreservation, such as of embryos and sperm, whenever a strain of mice is not in current use, to preserve unique alleles or allele combinations, and also to permit efficient export or rederivation of animals.

Whenever possible, we prescreen substances (including molecules to induce gene expression, cell death, mutagens, etc) and agents such as viruses *in vitro* to determine approximate doses required *in vivo*. When possible, we also test genome editing components *in vitro* (e.g. with ES cells), prior to the generation of genetically altered animals.
To maximise information gained from single animals, we use *in vivo* imaging when feasible, obtain data on as many tests of behaviour and learning and memory as possible on single animals, and obtain relevant tissue samples from multiple sites after killing. Where more than one project involves the study of an animal with a particular genotype, for example *Sox9* is relevant to studies on the CNS, pituitary and gonads, we often collect multiple tissues from single animals. Similarly, when designing new genetic tools, and maintaining animals derived with these, we will, wherever possible, do so in a way to allow them to be shared amongst as many people as possible, including making use of the host establishment sharing platform. This efficient use of animals minimizes the number used.

A retrospective assessment of reduction will be due by 22 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn

from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We choose well-established protocols, known to have minimal harmful effects, whenever possible.
Although it is not always possible to predict the nature or severity of any defect that arises from a newlygenerated genetic alteration, we take steps to minimise unwanted phenotypes and/or the number of animals exhibiting these. For example, we make use of tissue-specific regulatory elements and whenever practical, we prefer to make genetic alterations that are inducible, so that the animals do not show a phenotype until expression of the candidate gene or a deletion is induced. Animals exhibiting unexpected or detrimental phenotypes will be killed by a Schedule 1 method, or in the case of new lines or individual animals with phenotypes that may be of particular scientific interest advice will be sought from the Home Office Inspector.

When the experiment is predicted to lead to harmful effects outside the body system under study, we will provide treatments designed to alleviate these – for example, high salt will be given after adrenalectomy, and calcium lactate will be after removal of thyroid and parathyroid organs, or if tumour formation is not a desired outcome, then it may be possible to give anticancer agents (or growth inhibitors). By introducing substances, including viruses and cells, into specific tissues or cavities (such as the lateral ventricles of the brain) we minimise suffering because other body systems are not affected.
To minimise stress during breeding and maintenance we follow best practice guidelines, institute refinements and, for some strains, our own specific procedures of husbandry. These include cage enrichment, sufficient nesting material, and, for particularly sensitive strains and animals subject to specific procedures, minimum disturbance. In the case of any new strain of animal or application of any new procedure or refinement we pay special attention by increased observation and monitoring until we have become familiar with the phenotype and/or the consequences. If welfare implications are identified they will be acted upon and refinements considered in consultation with the NVS, NACWO and animal technicians.

Why can’t you use animals that are less sentient?

A significant fraction of our research involves studies on mouse embryos prior to two-thirds through gestation. We also make of chick embryos (covered under a separate PPL), for some projects. This is partly for evolutionary comparisons, and in some respects the chick and human may be closer than the mouse is to human (in morphology of the foetal ovary or as a model for the craniofacial abnormalities associated with mutations in *Foxl2*), and partly because certain embryological techniques are feasible *in ovo*, but not in utero. However, in most cases the mouse is a better model for the human situation, and the wide range of methods and tools that have been developed for the mouse makes this a more tractable model to study. In addition, many of the systems we study including sex determination, reproductive biology, the pituitary, and the CNS have aspects that are specific to mammals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For all manipulations, we will adhere to the relevant guidelines that aim to minimize suffering. We examine the animals for signs of pain and discomfort (such as grimacing), providing additional analgesia if appropriate, and monitor body condition, killing the animals if the distress is likely to be more than temporary. Many of the genetic and physiological manipulations, as well as the administration of substances, including gene inducers and repressors, viruses, cells and grafting of tissues, are standard and previous refinements from the literature will be used and added to if possible. For novel types of manipulation, or where insufficient information is available, small-scale pilot experiments are conducted in order to determine the best conditions to obtain a sufficiently robust and meaningful response from the minimum dose, exposure time or treatment. These pilot experiments help to minimize any potential suffering.
In all surgery, analgesia will be provided according to best current practice and with advice from the NVS/NACWO. Appropriate aseptic surgical techniques, heat, and fluid therapy, will be applied as necessary. For studies involving tumours, we will check the animals every day and kill any that exhibit signs of significant illness. Where possible, we will also use imaging methods to monitor the growth of tumours.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

These will include publications from the NC3Rs and the Institute for Animal Technology, but also relevant articles in scientific journals. In the case of cancer models, we will follow the guidelines in Workman et al, British Journal of Cancer (2010), 102, 1555-1577 (PMID: 20502460); or any subsequent updates as appropriate.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay up to date via regularly communication with animal facility staff at the host establishment, other scientists in our fields, via e-mail and other updates and publications from, and occasional attendance at meetings held by, the NC3Rs, the Institute for Animal Technology, and the International Society for Transgenic Technology, and through regular visits to their websites: https://www.nc3rs.org.uk/3rs-resources https://www.transtechsociety.org/ https://www.iat.org.uk/

A retrospective assessment of refinement will be due by 22 January 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

52. Energetics and homoarginine in heart & metabolic diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Heart failure, Ischaemia, Diabetes, Metabolism, Therapy

Animal types Life stages

Mice	adult, neonate, juvenile, pregnant, aged
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to further our understanding of energetic pathways and the interplay between creatine and homoarginine in the heart and other metabolic tissues. This includes generating proof-of-principle evidence in rodent models in support of new therapeutic approaches for ischaemia and chronic heart failure.
A retrospective assessment of these aims will be due by 26 February 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In the UK alone there are ~100,000 heart attacks per year and 800,000 individuals living with heart failure. Even with optimal treatment, 59% of men and 45% of women diagnosed with heart failure will be dead within 5 years, a clear indication that new and better treatments are urgently needed.

What outputs do you think you will see at the end of this project?

The major output of this programme of work will be the generation of new information that will advance scientific knowledge, in particular, how creatine and homoarginine interact and affect whole body metabolism, ischaemic disease, diabetic cardiomyopathy, and chronic heart failure (Objective 1). We will also provide proof-of-principle evidence for therapeutic potential using appropriate disease models, with the aim of moving closer towards clinical translation to humans (Objective 2).
The outputs will be evidenced by publication in international peer-reviewed scientific journals and by presentation at scientific conferences. Other outputs may include the characterisation of drug-like molecules that we would consider patenting and could be used by others as a starting point for the development of new medicines.

Who or what will benefit from these outputs, and how?

The immediate beneficiaries will be the scientific community, who within the time-frame of this PPL, will gain new information that can be used to guide future work and will lead to the gradual accumulation of scientific knowledge in this area. This knowledge is also likely to be useful to the medical community and pharmaceutical industry since our research aims to answer key scientific unknowns that will bring us closer towards clinical translation to humans. By way of example: -
Our finding that homoarginine preserves contractile reserve in mice with heart failure already has potential for direct translation to benefit patients, particularly since hArg is cheap, can be taken orally and is safe in humans. Within the next few years we will seek funding for a clinical trial in heart failure patients. However, the work in this PPL will remove barriers to translation by addressing unknowns such as optimal dosing and a detailed understanding of mechanism in the heart and other organs, thereby paving the way for these trials. In the long-term (7-10 years), as an add-on to standard therapy, homoarginine has the potential to improve the quality of life for patients with chronic heart failure.

We have identified augmentation of creatine kinase activity or myocardial creatine levels via activation of the creatine transporter as a potentially beneficial strategy in diseases of ischaemic origin. The largest obstacle to translation is a lack of pharmacological tools to test this strategy in other disease models and as lead compounds for drug development, hence we are currently collaborating with the pharmaceutical industry to remedy this. By testing new compounds, or equivalent genetic modifications, in relevant disease models we will provide proof-of-principle evidence for future translational studies. By including co-morbid models of diabetes, we will ensure these benefits are also realised in the diabetic heart. In the longer term (10 years), this will move us towards the realisation of clinical benefit, e.g. for cardiac protection during heart surgery, treatment of angina and peripheral vascular disease, and diabetic cardiomyopathy.

How will you look to maximise the outputs of this work?

We aim to disseminate our findings as widely as possible to the scientific and medical communities via publication and presentation at conferences. Outputs are maximised by ensuring our research is conducted and reported with openness and rigour, with the aim of creating a legacy of trusted and reproducible new knowledge. This includes the publication of unsuccessful approaches, so that others can learn and concentrate resources on what works best without unnecessary duplication. We will actively engage in informed debate by authoring review articles, by giving seminars, and by public engagement.
We will seek opportunities to collaborate with other scientists and medical professionals in order to identify areas of synergy that will further our objectives, expand our findings into new therapeutic areas, or open new areas for investigation. For example, collaboration with colleagues that are expert in the design of clinical trials or drug development will be necessary to realise the long-term benefits of this project.

Species and numbers of animals expected to be used

- Mice: 16400
- Rats: 300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult mice will be used for the vast majority of this project because this species provides a unique combination of easy genetic manipulation with a cardiovascular system and disease models sufficiently similar to humans. Occasionally we will use rats, for example, because it is possible to induce coronary vasospasm under terminal anaesthesia as a refined model of angina in this species, or where a larger heart is required to make scientific measurements. We may also use rats to demonstrate that a new drug works the same way in different species, which would be necessary to justify more complex experiments in larger species such as pigs.

Typically, what will be done to an animal used in your project?

We will use surgical models that mimic the major causes of human heart failure since these are the most translational and therefore considered the gold standard in our field. A typical experiment might compare whether mice with a specific genetic alteration in the heart are better than control mice at responding to a heart attack. To do this, we perform surgery under general anaesthesia to stop blood flow in a coronary artery, thereby inducing a heart attack. The animal is given painkillers during recovery and we follow how the heart responds over the course of 6-8 weeks. This involves similar techniques to those found in a cardiology clinic, for example,

ultrasound or MRI imaging on one or two occasions to measure heart function and structure, and placing a catheter in the heart to directly measure pressure generation. All of these are performed under general anaesthesia and to control cumulative suffering the maximum number of anaesthetics in a lifetime is limited to 8. At the end of the experiment the mice are killed humanely and the heart removed to study the molecular and cellular changes.
Other variations of this experiment may use mice or rats that are being treated with a novel drug that we hope will be beneficial in heart failure. In this case we will give the drug by the oral route whenever possible, but if not, this may require daily injections. We will perform similar experiments using other models of heart failure, e.g. due to narrowing of the aorta or secondary to diabetes.
Mice will be made diabetic by one of the following methods: daily injections for 5 days with a compound that disrupts insulin secretion; feeding a Western-style diet to make them obese; or they will harbour genetic mutations that lead to diabetes. Experiments will last several months to allow for development of diabetes and cardiac complications during which time they will have urine and blood samples taken regularly to monitor glucose levels. At the end of the experiment we will measure heart function by ultrasound and cannulation for pressure measurements. Some of these animals will be given a heart attack under general anaesthesia as a final step, after which they will be killed humanely.
Approaches that are beneficial in the heart will also be tried in a model of skeletal muscle ischaemia, where blood flow to one of the legs is restricted to mimic peripheral vascular disease in humans. This involves surgery to occlude one of the main arteries supplying a hind-limb (the other limb acts as a normal control). An experiment may last up to 3 weeks post-surgery during which mice may receive test compounds and housed singly with access to a voluntary running wheel to measure exercise capacity. They will be anaesthetised on 1 or 2 occasions to non-invasively measure blood flow in both legs before being killed humanely to collect tissues for further analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

We will use surgical models of heart failure and many of the adverse effects are related to recovery from the surgical procedures used to create the disease models, e.g. pain, weight loss, poor body condition. However, these can mostly be controlled via good practice, for example, surgery takes place using aseptic technique within a clean air environment, animals are closely monitored during recovery and provided with pain killers, fluids, heat support and access to softened food. Most recover well within a couple of days and will remain free of adverse effects for the duration of the protocol. However, over subsequent weeks, approximately 10% may develop shortness of breath or laboured breathing, which is indicative of congestive heart failure. We monitor mice frequently to identify these symptoms and humanely kill affected animals at the earliest opportunity. Nevertheless, some animals will die of heart failure since these symptoms can develop rapidly.
In some models of diabetes the mice will lose weight, while in others the diabetes is secondary to obesity. In both cases, the mice may show poor body condition, will feel thirsty, and urinate more. Absorbent bedding, extra water and cage changes are utilised to prevent the potential for developing skin sores.
Although mice will develop measurable heart disease, this will not be severe enough to cause clinical symptoms.
The model of peripheral vascular disease utilises the same good surgical practice outlined above. Mice are given painkillers during recovery and will typically limp for a few days while their leg adapts. They will always have one good leg and are therefore still mobile and able to feed for themselves. Strength will progressively return to the affected limb over the course of the experiment. Use of a running wheel necessitates single housing, which may be stressful to the mouse, however it is partly mitigated since mice enjoy running on a wheel.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of mice will not experience adverse effects because they will be used for breeding purposes in order to establish and maintain animals with specific genetic alterations. Approximately 15% of these lines may have a harmful phenotype of moderate severity, e.g. they will be underweight with muscle weakness or obese with early signs of diabetes. Animals that have surgery but otherwise recover well are also considered moderate severity, since the adverse effects are well controlled and of short duration. However, the <10% of animals that develop congestive heart failure might have a severe experience, since they may have difficulty breathing for several hours before being found dead or euthanased. There is also potential for cumulative suffering leading to a severe severity, e.g. where the same animal receives multiple procedures as part of a single protocol. To mitigate against this, animals are allowed to fully recover before the next step in a protocol and we have limits on the total number of general anaesthetics administered in a lifetime.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 26 February 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are studying complex diseases such as chronic heart failure, which is a progressive disease that develops over many weeks. This represents a level of complexity that can only be fully represented by the intact animal since there is dynamic interplay between mechanical stress, haemodynamic loading, and the nervous, vascular, endocrine and inflammatory systems.

Which non-animal alternatives did you consider for use in this project?

We use non-animal alternatives whenever possible and consider these as complementary to the animal work, i.e. they may reduce the number of animals required, but do not completely replace the need for subsequent animal work. For example, we are running an *in vitro* screen to discover new drugs using a cell culture assay, however, any positive hits from this will ultimately need to be tested *in vivo*. We routinely perform many *in vitro* experiments using cell culture, since these are particularly useful in determining the consequences of altering gene expression at a molecular and cellular level.
We have an active collaboration with clinical colleagues to study energy metabolism in human heart disease. This includes access to small amounts of human myocardial samples and we also have access to a biobank of human adipose tissue that will allow us to validate our findings and show that they are relevant to human disease.

Why were they not suitable?

Human tissue is a valuable and useful resource, but the quantities obtained are very small, the samples exhibit a lot of variability, and it represents a snap-shot of disease. Taken together this greatly limits the types of experiment it can be used for.

We have previously used computer modelling for certain aspects of our research. However, when we tried modelling the effect of altered creatine levels on heart metabolism, we found that the computer model could not predict the widespread and varied metabolic response we have observed in animal experiments.
Homoarginine is our other metabolite of interest, but it has not yet been incorporated into any computer models, since it is unknown what proteins and pathways it interacts with. This project aims to address this gap in our scientific knowledge.
A retrospective assessment of replacement will be due by 26 February 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Breeding will make use of the majority of animals in this project and we have based our numbers on usage over the previous 5 years, since we anticipate the overall workload to be similar. We also know from experience that a fully controlled study in our heart failure models requires ~100 mice and will take ~1 year to complete, so 500 mice over a 5 year period is the maximum we anticipate using. In practice, the protocols we use will be guided by our scientific findings, so it is highly unlikely that we will use the maximum numbers across all protocols.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We routinely use power calculations to guide our experimental design. This is a statistical technique that provides an estimate of how many animals are required in each experimental group based on the anticipated effect size and the variability observed in previous experiments.
We plan to make good use of non-invasive imaging techniques, such as ultrasound and MRI, which allow repeated measurements to be made in the same mouse at multiple time-points. This significantly reduces the number of mice required for longitudinal studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

By careful advance planning, we aim to closely match our experimental requirements with breeding output, and thereby avoid wastage. Our institution has a dedicated mouse colony expert who provides advice on efficient breeding. We freeze embryos or sperm from mouse lines that are not in routine use, since this avoids the need to breed mice simply to maintain a live colony. Wherever possible we will share spare tissue with other groups that can use it. For example, we do not study the brain, but our collaborators in Germany do, so we freeze organs from our experiments that may be of use to others.
We make use of pilot studies when working with new drugs or a new mouse line. Initial study in a small number of animals provides information on adverse effects and the natural progression in our disease models. We can then adjust the monitoring, experimental and humane end-points accordingly.
A retrospective assessment of reduction will be due by 26 February 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mostly mice for this project because of the ease of genetic manipulation and the similarity of the cardiovascular system to humans. We will use surgical models of heart failure, because these recapitulate the complexity of human disease and the discomfort associated with surgery can be mitigated (as detailed below). Alternatives include infusing drugs to cause heart failure, but the overstimulation of a single pathway does not reflect any common cause in patients, and mice are still at risk of developing congestive heart failure, which is the single biggest welfare concern. We therefore believe that the heart failure models used in this project are the most clinically relevant that also cause the least suffering to the animals.
We will use mouse models of diabetes in order to study diabetic cardiomyopathy and the early response to heart attack. These include injection of streptozotocin or high-fat diet or genetic models of obesity to reflect different types of diabetes. We need these models to develop heart dysfunction when measured by ultrasound, but they are less likely to develop clinical symptoms of shortness of breath compared to the surgical models of congestive heart failure. The major clinical symptoms are therefore related to changes in body weight and elevated blood glucose
We will generate a model of peripheral artery disease (hind limb ischaemia) by surgically tying-off an artery in one leg. The other leg is left unaffected, so although mobility is impaired in the first few days, animals will always be able to move around the cage and feed normally. The alternative model removes the entire artery and surrounding vessels and is associated with a high level of foot necrosis (up to 25% compared).
We will use a range of non-invasive methods to measure the effect on the heart and other organs, e.g. electrocardiogram, ultrasound, MRI, laser Doppler, relaxometry (body composition), blood sampling, and voluntary wheel running. These are all particularly benign and produce a wealth of scientific data without causing distress or lasting harm to the animals.

Why can’t you use animals that are less sentient?

We are studying how the heart responds over time to injury and the complex interplay of haemodynamic forces, energy requirements and regulatory systems. The human heart, in common with other mammals, has four chambers and generates normal pressures of 100-120 mmHg. Fish can be used to study acute heart injury and healing, but they only have two chambers and generate 2-3 mmHg in pressure, while frogs have a 3-chamber heart and generate 30 mmHg. Mice are therefore the least sentient species that have a cardiovascular system and response to disease that is sufficiently similar to humans. We need to use adults, because most heart disease affects adults.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

When genetically-altered mice exhibit a harmful phenotype it is usually possible to conclude our experiments at an earlier time-point in order to minimise potential suffering or distress.

We will use disease models that have direct correlates to the major causes of human heart failure, e.g. ischaemic (following a heart attack), pressure overload (aortic stenosis), and diabetic cardiomyopathy. The first two of these are surgical models with the potential to cause pain and suffering during recovery, however, this will be mitigated by using aseptic technique and giving pain killers, fluids, softened food, and heat support during the recovery period. The majority of mice make a full recovery within days and will not experience further adverse effects. In the ischaemic model there is a risk of sudden death due to scar tissue rupturing, but this affects mostly males, so we typically use females for these experiments. Around 10% of mice will develop shortness of breath or laboured breathing during the following 6 weeks, which is indicative of congestive heart failure. Suffering is minimised by increased monitoring and using this as an immediate humane end-point.
For diabetic mice, we will regularly monitor blood glucose levels and humanely kill animals if they become dangerously high. We expect the mice to be thirsty and urinate more, so we will make use of absorbent bedding and keep fewer mice in each cage.
For mice with hind limb ischaemia, the same controls as above will mitigate against pain and discomfort caused by the surgery, and after a brief spell of reduced mobility, mice usually recover well.
Regular monitoring will identify animals that do not recover mobility or that develop early signs (e.g. skin discolouration or nail injury) and these will be humanely killed.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow Home Office guidance on the “Code of practice for the housing and care of animals bred, supplied or used for scientific purposes”. Guidance for aseptic surgery will be taken from “Guiding Principles for Preparing for and Undertaking Aseptic Surgery” (LASA 2017). At the experimental planning stage we will refer to the PREPARE guidelines checklist (“Planning Research and Experimental Procedures on Animals: Recommendations for Excellence”) and to ensure our experiments are reported effectively we will adhere to the ARRIVE guidelines (“Animal Research: Reporting of *In Vivo* Experiments”).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

At the start of each experiment we will check 3Rs websites such as NC3Rs (https://www.nc3rs.org.uk/3rs-resources) and Norecopa (https://norecopa.no/3r-guide). We also receive regular newsletters from these and other organisations, our department holds animal welfare meetings three times a year where progress on the 3Rs is discussed, and there is a regular institution-wide newsletter on the 3Rs.
To keep abreast of new applications we actively scan the scientific literature for alternatives and talk to other researchers that are performing similar techniques to establish best practice. Hands-on advice from the vet is always available in implementing advances effectively.
A retrospective assessment of refinement will be due by 26 February 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

53. Environmental influences on immunity and tissue biology

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

mucosal immune system, intestinal stem cells, environmental signals, cancer, infection

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To study how environmental triggers transmitted via the aryl hydrocarbon receptor affect immunity and tissue biology

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

While it is known that genetic traits contribute substantially to how the immune system works to protect against pathogens and tissue damage, it is equally clear that environmental factors strongly impact such responses. Our studies have identified a protein which acts as an environmental sensor, the aryl hydrocarbon receptor (AHR). AHR functions as a molecular entry point for environmental factors that can be man-made pollutants (xenobiotics) or can be derived from the diet or the microbiota. Stimulation of AHR with dietary ligands helps to maintain the integrity of the intestinal barrier, through effects on different immune cell types as well as tissue cells such as epithelial and endothelial cells (which are the cells lining the gut or the vessels). We are working on the underlying mechanisms to understand why dietary AHR ligands are beneficial whereas pollutants have detrimental effects on health.

What outputs do you think you will see at the end of this project?

At the end of the project we anticipate to have a greater understanding of how environmental factors transmitted via the AHR influence the immune system and tissues in steady state as well as after challenges such as infections, tissue damage or the growth of tumours. We will disseminate our results in conferences and publications as well as in public engagement events.

Who or what will benefit from these outputs, and how?

Since many of the natural AHR ligands are derived from the diet, it is possible that dietary supplementation can be used for preventative purposes or for alleviation of inflammatory reactions particularly in the gut. In the long term this might be applicable to humans with intestinal disorders or the genetic predisposition to develop such disorders.

How will you look to maximise the outputs of this work?

We are collaborating with toxicologists who focus on the detrimental effects of pollutant AHR ligands to understand the underlying reasons for the different outcomes following exposures to these compared with dietary AHR ligands. We also have collaborations with groups that work on infection of the lung as AHR seems to play a role in tissue repair following infection. We have written several comprehensive reviews on the role of AHR in the intestine and other tissues and I am frequently asked to give seminars or conference presentations on our research.

Species and numbers of animals expected to be used

- Mice: 30000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice genetically modified to have deficiencies in the AHR pathway in different cell types using gene editing methods in order to evaluate where AHR is needed to protect against infection, inflammation and tumorigenesis. In most of our mice AHR is deleted or altered genetically so that the mice express these altered genes from birth. However, we also will use methods to be able to induce deletion or alteration at certain life stages. This is particularly important for assessment of AHR in cells lining vessels (endothelial cells) as the existing literature suggests that AHR is influencing vessel development very early in life. For this reason, we will need to use some neonates and juvenile mice to evaluate the consequences of AHR deletion early in their life, compared with deletion in adult stage. We anticipate that AHR roles early in life may be recapitulated in adult mice upon injury which requires restoration of vessels.

Typically, what will be done to an animal used in your project?

In a typical scenario animals might be subjected to administration of substances to induce gene deletion and/or activate or inhibit AHR. They might then be infected with a pathogen . Following such treatment the mice might be subjected to imaging under anaesthesia, they might have blood taken for analysis (and will finally be killed typically about 4 weeks after Step 1, which may involve perfusion or exsanguination under terminal anaesthesia. A minority of animals (<10%) might in addition experience irradiation followed by bone marrow reconstitution or transfer of immune cells as well as treatment with antibiotics. Protocol 5 (Induction of colon tumorigenesis) will follow mice over 12-16 weeks as induction of tumorigenesis, especially in wildtype mice is a slow process.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals may experience pain, weight loss and tumours for a short period of time

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Immunocompromised mice and those lacking AHR might be more susceptible to the procedures and reach the moderate severity limit of the protocols.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

While it is possible to generate information regarding regulation of some aspects of the immune response in cell culture it is not possible to mimic the response to infection or intestinal disease in vitro, since immune cells are highly connected with cells of the tissue lining and crosstalk between different cell types is an essential feature of inflammatory responses.

Which non-animal alternatives did you consider for use in this project?

We will where possible, collect and generate as much data as possible using culture methods for immunological tests. For instance, the state of intestinal epithelial cells in genetically modified mice may be investigated using organoid cultures which are often termed mini guts.

Why were they not suitable?

In vitro systems, while useful, do not fully replicate the complexity of immune interactions or disease pathogenesis in vivo and it is essential to use appropriate and robust animal models to dissect these processes. Furthermore to develop therapeutic approaches with potential to alleviate human disease it is necessary to establish parameters influencing efficacy in an animal model.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For the design of most of the quantitative experiments, sample sizes will be set using power analysis, generally using a significance level of 5%, a power of 80% and at least practicable difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own or from the literature). Wherever possible, factorial design will be employed to maximise information using the minimum mouse number. Statistics expertise will be sought within the institute, and active statistical discussion with bioinformaticians. Pilot experiments will use between 5-8 mice per group, which should be sufficient if a significant result is obtained. Experiment will be repeated to obtain further significance if there are only small differences; in this case it may have to be repeated with larger numbers of mice and/or modifications.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Measurements over time, in particular non-invasive ones, such as weight loss, clinical scores and non-invasive imaging, allow gaining a wealth of information on disease course over time with minimum number of mice used. Freezing of mouse embryos, tissues and cells is routine at the establishment and will ensure that the minimum number of mice is bred, and measures are in place to maximise efficiency of breeding schemes with minimum surplus. Reporting will be based on ARRIVE guidelines. Imaging technology will allow following a cohort of mice over time rather than setting up several experimental groups to monitor the consequences of an infectious stimulus. Furthermore, colonoscopy with an endoscope will be a monitoring method for the colitis and tumorigenesis protocols to allow us to detect inflammation and tumorigenesis earlier and without the use of large cohorts of mice. We will remain alert to any advances, which will enable the replacement of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The efficiency of animal usage is maximised in consultation with animal technicians, by careful control of breeding to meet research needs with respect to numbers, the reproducibility of their responses and health. This has been greatly facilitated by a mouse database in which every breeding pair and every mouse born are recorded and through which we can readily monitor the numbers of mice we hold. Littermates which do not express the modified genes, i.e. are wild type for the respective gene(s) from the breeding protocol will be used as appropriate age and gender matched controls. This allows optimal use of mouse numbers generated and is best scientific practice for the study of genetic alterations.

For experiments on tissue inflammation or the development of cancer in response to infection we will wherever possible make use of imaging to follow a cohort of mice over time, which will substantially reduce the number of mice involved. Lastly, this programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. This highly integrative approach will maximise the information obtained from the minimum resource.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The models of infection, tissue inflammation and tumorigenesis are the best-established models in the field with high relevance to human disease states. They are generally non-invasive and are likely to

yield significant results without causing lasting suffering and pain.

Why can’t you use animals that are less sentient?

The mouse model allows for control over host genetic factors, which is crucial to studying the factors responsible for development of disease. With these models we can investigate how the immune system recognizes and responds to infection, how tissue damage is repaired and how tumours arise which can lead to new avenues of treatment and prevention.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will work closely with the veterinary staff to ensure that we are always refining our protocols to minimize harms for the animals we work with.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will stay up to date with the best practice guidelines developed by the National Centre for the Replacement, Refinement, & Reduction of Animals in Research, and the scientific literature for estimation of sample sizes based on power calculations.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We keep up with the latest developments in the field by reading the relevant literature and we have ongoing discussions of 3R measures in the institute which ensures we are always up to date.

Home Office

NON-TECHNICAL SUMMARY

54. Evaluation of Novel Strategies for Bone Repair

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Rats	adult
Rabbits	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project will determine the safety and effectiveness of different natural and synthetic materials, alone and in combination with cells and/or adjunct treatments, that might be used to enhance the healing of bone.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Millions of patients each year develop problems secondary to incomplete healing of fractures caused by trauma or disease (e.g. cancer, osteoporosis). Fracture complications negatively affect the patient's quality of life, with prolonged hospitalisation, potentially crippling disability and, in extreme cases, infection and a risk that the limb may be lost. The average healthcare cost for managing a non-healing fracture has been estimated to range from £17,000 to £79,000 per patient.

There are a number of reasons why fractures fail to heal normally. First, the amount of bone lost as a result of the original trauma or disease may be too great, exceeding the ability of the skeleton to repair itself. The second major reason is that the patient's bone may not be healthy – this is common in elderly patients and in those taking certain medications. Another important reason for fractures failing to heal is that the bone may be infected, either at the time of the original injury or at the time of surgery.

Whatever the underlying cause, there are currently two main ways to replace bone and stimulate healing in patients with these problematic fractures. The first relies on bone that is removed from the patient him/herself, then placed into the site that is not healing. The use of the patient's own bone – so called "autograft" – is relatively easy from the surgeon's perspective – the bone is immediately available (it is usually harvested from the pelvis of the patient) and since it comes from the same patient, there is no risk of disease transmission or of adverse immune responses to the graft ("rejection"). However from the patient perspective autograft bone suffers from some important limitations. For instance collection of the bone graft requires a second incision, which causes additional discomfort for the patient following surgery. Additionally, there is a physical limit to how much bone can be harvested from the pelvis, especially in children and in smaller adults. Finally, the quality of the bone that is collected can be quite variable, especially if the patients are being treated with chronic doses of medications such as steroids, which are known to reduce the biological properties of autograft bone.

As an alternative to using the patient's own bone, the surgeon can elect to use bone that is harvested from cadavers. This bone – known as allograft – is typically obtained through commercial tissue banks. While quite plentiful, allograft can be very expensive to buy. Additionally, because it comes from a different person, there is potential for transmission of infectious diseases such as HIV, Hepatitis B and Hepatitis C. Donors are screened for these diseases, but a small risk is always present.

There is tremendous interest in the development of safe, effective and reasonably-priced alternatives to human tissue for enhancing bone repair following bone loss secondary to trauma or disease. Given the concerns over both availability and potential disease transmission from natural human bone products, there is a necessary drive to develop alternatives to human bone, including the use of non-human (xenograft) bone or bone derivatives, synthetic biomaterials, or combinations of natural and synthetic materials.

We and other groups are actively working to develop these so-called bone graft replacements. In most cases, these are based around either chemically processed forms of native bone, synthetic forms of bone mineral (typically derivatives of calcium phosphate), or some form of natural or man-made polymer (common examples include collagen, poly-lactic acid (PLA) or poly-caprolactone (PCL)).

Our lab has focused in particular on the development of a chemically processed form of bovine bone that might be suitable as a building block for making a bone graft replacement. We elected to focus on bovine bone because of its widespread availability (via the food chain) and established history as an implantable biomaterial (including both soft tissues such as heart valves, and as bone and tendon grafts). Bovine bone has similar macrostructure and microstructure to human bone (more comparable than is seen in sheep and goats, for example) and displays Haversian (osteonal) remodelling. It also has excellent material/mechanical properties that are well aligned with those of native human bone. By combining the processed bone with a resorbable polymer, we will be able to create a bioresorbable patient-specific implant with mechanical properties that are specifically tuned to meet the demands of the site in which it is implanted. The material is amenable to being fabricated into complex structures through the use of 3D printing techniques. Laboratory testing of the processed bone has shown that it is well tolerated by bone cells, and that it its mechanical properties are similar to those of native bone. In order to explore its potential effectiveness in healing bone, we now propose a series of animal studies that will establish whether (a) there is any evidence of toxicity or adverse tissue response when the material is implanted in animals, and (b) whether 3D printed cylinders of the material can serve as a framework, or scaffold, for bone repair.
It is critically important that any new candidate material performs at least as well as currently approved products in vivo - otherwise there is no value to the new material.
This project license describes a series of rodent and rabbit studies that will allow for robust, systematic screening of a range of candidate materials (biomaterials) as scaffolds for bone repair. In addition to testing the safety and effectiveness of these biomaterials as stand-alone scaffolds, we will also establish whether it is possible to further enhance bone healing by adding stem cells to the material prior to implantation, or by using post-operative treatment with biophysical stimuli such as shockwave therapy to see if this further enhances the reparative capacity of the scaffold-cell combination.

What outputs do you think you will see at the end of this project?

We expect to publish a minimum of 2-3 scientific papers reporting the results from the work under this license. These papers will describe the development of a new type of bone repair material. At the same time, positive results from these animal studies would likely lead to interest in the development of a commercial product for use in the veterinary clinic (for repairing fractures in cats, dogs and horses). With time, there may then be interest from the human market for a product that could be used in orthopaedics, dental surgery and possibly spine surgery.

Who or what will benefit from these outputs, and how?

We anticipate direct benefit to veterinary and human clinical patients who require surgical management of bone defects. As part of the work, we will validate both the rat and rabbit models for bone defect studies and our findings (and subsequent publications) should be helpful to other researchers who might be considering similar work in the future.

How will you look to maximise the outputs of this work?

We are working to establish collaborations within our institution, as well as with collaborators at other scientific centres around the world. These collaborations will benefit from the data developed under this project license.

The animal models described in this project license will be published and we would expect this to lead to more general adoption of these models in the future.

Species and numbers of animals expected to be used

- Rats: 120
- Rabbits: 324

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rats and rabbits are widely recognised as appropriate preclinical screening models for determining the tissue response to orthopaedic, dental and spinal implants. Regulatory bodies that oversee the testing and approval of medical devices recommend the rat and rabbit as appropriate models. The rat model involves a relatively small bone defect and is therefore ideal for screening a lot of different possible materials. The rabbit model involves a much larger and more clinically relevant bone defect that is better suited to providing more detailed information about the materials that perform best in the rat model. We use skeletally mature animals because most of the patients that we see with complex fractures or tumours are adults.

Typically, what will be done to an animal used in your project?

For the surgical models, the common procedures will be anaesthesia, surgical implantation of one or more biomaterials, then follow-up for periods of up to 6 months. For the subcutaneous model in rats, small cylinders of the test material will be placed under the skin. For the rabbit model, a bone defect will first be created by removing a 15-mm length of bone from the femur; this defect will be replaced with a cylinder made from one of the test materials, and the bone will be stabilised with a metal orthopaedic plate and screws. At intervals after the surgery, chemical dyes that mark sites of active bone formation will be injected into the animals so that we can measure bone healing. In some animals, additional treatments (including the use of shockwave treatment to stimulate bone repair) will also be evaluated. None of the animals will have more than one surgery to implant the materials, and for the bone defect models only one leg will be operated.

What are the expected impacts and/or adverse effects for the animals during your project?

We expect to see some weight loss as a consequence of interruptions to normal feeding patterns. For animals undergoing orthopaedic surgical procedures, it is very likely that there will be some degree of lameness secondary to the bone surgery – this is what we see in clinical patients undergoing orthopaedic surgery. We will use clinically approved pain-relieving medication to minimise pain and discomfort after surgery. The risk of infection - also ever present in surgical patients - will be reduced by the use of aseptic technique, sterile instrumentation and appropriate wound monitoring and topical wound care. It is not our routine clinical practice to use antibiotics for elective orthopaedic surgery, so antibiotics will not be used routinely in rats and rabbits on this project.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All of the rats on this license are expected to fall under the moderate category as they will all undergo surgery. The rabbit procedures are also considered moderate severity. That said, we anticipate that the overall impact of surgery will be less for rats than for rabbits - the implants in rats are placed in the tissues immediately under the skin, while those for the rabbits are placed directly into bone. We do not expect to see lameness in rats where we will see this in the rabbits.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are needed to explore the specifics of the tissue responses that happen following implantation of a biomaterial. Biomaterials are natural or synthetic materials that are designed to replace or augment an organ, or a bodily function, or to stimulate natural tissue repair. For this project, we are focusing on biomaterials that will improve the body's ability to make new bone. All biomaterials that are intended for clinical use in human or veterinary patients must first undergo animal testing to ensure that they are safe and effective. The material that we have developed is novel and has not been tested previously. As such, there is no way to secure approval for clinical use without screening the material in an animal model.

Which non-animal alternatives did you consider for use in this project?

The preliminary work underpinning this project has been performed exclusively using cells growing in the lab. The biomaterials that we have identified as being potentially useful must first be shown to be safe for normal cells. We then go on to test the mechanical properties of the different biomaterials to ensure that they are strong enough to survive in a bone defect environment. None of this preliminary work involves the use of animals, and only biomaterials that pass all of these early tests are considered for animal testing under this project license.

Why were they not suitable?

Although cell culture and lab-based models can be very helpful in exploring the effects of different compositions on biological and material properties, they do not and cannot address fundamental questions regarding the interactions between the material and the tissues in which it is implanted. For example, bleeding at the surgical site may interfere with the integration of the material into bone, while immune reactions stimulated by foreign cells or proteins may lead to inflammation that could compromise new bone formation and integration. Additionally, the rate at which the polymeric (plastic) component of a biomaterial is degraded cannot be determined without testing in animals – if the material disappears too soon the mechanical integrity of the structure may be compromised, while if it too slow it may adversely affect new bone formation. The use of clinically relevant animal models allows us to perform temporal studies that can address these interactions and compare the performance of the new material (in its different compositions) against that of materials that are already in clinical use. Current options for bone replacement in animals and humans exist but all have limitations in terms of cost, availability and flexibility when used in different applications. The materials that we are exploring offer substantial advantages in terms of being cost-effective, safe and adaptable to the patient's unique anatomy (through the use of 3D printing based off CT or MRI scans).

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We initially estimated the numbers from a review of previously published data from the rat and rabbit models, which indicate sample sizes of anywhere from N=8 specimens per group (for standard rat subcutaneous models for assessing tissue response to biomaterials) to 10-15 specimens per group (for rabbit bone defect models in which biomaterials are placed into long bones).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We undertook a number of steps ahead of submitting this project application, including:

1. Detailed literature review to look for alternatives and best practices to reduce overall animal numbers

2. Consultation with NC3Rs Experimental Design Assistant to ensure that we are maximising data collection from every animal

3. Sequential design of experiments so that treatments that are found not to be successful in rats are eliminated from further consideration and do not go into rabbits.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Steps to optimise animal use include:

1. Use of shared control groups across the different phases of testing.

2. Use of non-invasive imaging (such as X-ray and CT scans) and non-destructive tests that allow specimens to undergo mechanical testing and still remain sufficiently intact to be useful for biochemical or histological studies. By doing more than one set of measurements on the same specimen, we are better able to make direct comparisons between outcome measures.

3. Use of injectable chemical dyes (fluorochromes) to measure bone formation at different time points in the same animal.

These dyes stain the bone and provide a time stamp - by looking at the pattern of this staining (with a microscope) after the animal is dead, we can retrace the history of bone healing in the animal...rather like examining tree rings and establishing periods of relative drought and relative plenty. Multiple labels can be injected in each animal, allowing us to compare healing patterns at different time points, without the need to kill animals at individual time points.

4. Compliance with PREPARE and ARRIVE guidelines related to the design, conduct and reporting of animal studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain

management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will first start by establishing that the combination materials (composites) are safe (non-toxic) when implanted in subcutaneous sites in rats. The subcutaneous model, with implantation on the dorsum of the rat, is well tolerated, with transient pain/distress that is similar to that seen with repair of a simple surgical incision.
Material and material-cell combinations that demonstrate acceptable safety will then go on to testing in a clinically relevant bone defect model in rabbits.
The rabbit bone defect model provides a clinically challenging environment for bone repair. The instrumentation used to stabilise the femur is strong enough to protect the bone even if the candidate material is ineffective at supporting new bone growth, so we do not expect to see a significantly higher rate of complications in animals that are implanted with biomaterials that prove not to be effective.The alternative to the rabbit would have been the sheep - this would have offered the opportunity for an even larger bone defect, but we felt that this fact alone was insufficient to justify moving up to this species at this time. If the candidate materials are especially promising and we are able to commercialise the material, regulatory bodies may subsequently ask for sheep data, at which time we would submit an amendment to this license.

Why can’t you use animals that are less sentient?

We cannot assess acute or chronic tissue reactions to implanted biomaterials in anything other than a live-animal model. Cadavers and terminal surgery are helpful in optimising surgical technique but can never replace the need for live animals in work of this type.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We leverage the skills of our technical and animal care teams to ensure effective monitoring of animals from both a behavioural and a welfare perspective. Pain-relieving medications are given routinely after invasive or potentially painful procedures, and validated pain scoring/behavioural scoring is used to confirm that pain relief is sufficient. Daily direct observations of the animal, and routine monitoring of body weight and food intake are used to ensure the welfare of animals in our care. When we can, we will train animals to allow for oral (or in-food) administration of pain-relieving medication. We have used shockwave therapy in clinical veterinary patients (dogs and cats) for over 2 years without any need for sedation, and we anticipate that the procedure will be well tolerated in rabbits without sedation. However, to ensure minimal stress we will use sedation or short-acting inhalation anaesthesia in this preclinical study. We use non-invasive and non-painful techniques for imaging, and minimise the number of interventions needed to secure a scientifically valid answer.
All animals will be group housed in order to ensure social interaction and normal behaviours (e.g. play and social grooming). Species-appropriate environmental enrichment will be provided (e.g. Cheerios and plastic shelters for rats; plastic balls for rabbits to play with).
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All of our experimental work is designed and performed in accordance with established best practices. These vary from the use of the LASA Guide, NC3Rs guidance, development of surgical standard operating procedures based on publications, use of validated pain scoring schemes, and the design and reporting of studies using PREPARE and ARRIVE guidelines, respectively.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I receive regular updates on developments in the application of the 3Rs through institutional communications and mailings from international animal care organisations such as the Association for Assessment and Accreditation of Laboratory Care International (AAALAC International). My lab is committed to using best practices in our work and would certainly institute advances in, for example, pain scoring and rabbit analgesia if demonstrated during the course of this project.

Home Office

NON-TECHNICAL SUMMARY

55. EVALUATION OF NOVEL THERAPEUTICS FOR TOXICOLOGICAL SAFETY

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Toxicology, Exploratory Toxicology, Regulatory Toxicology

Animal types	Life stages
Mice	adult, juvenile
Rats	adult, juvenile
Hamsters	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of the project is to assess the possible toxicity potential of novel therapeutics for clients/partners using well established regulatory or non-regulatory toxicology protocols in protected animals.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to generate scientifically valid toxicological data that can be used in determining the potential safety levels of novel therapeutics to progress them to regulatory work and to satisfy the regulatory authorities prior to potential therapeutic use in humans.

What outputs do you think you will see at the end of this project?

It is reported 10.3-27% of oncological patients experience toxicological adverse events in phase I-III clinical trials with a 0.4-2% mortality rate (Wheler J. J. et al., 2012; Witteles R.M. and Telli M. 2012). Toxicological adverse events are also observed with other pathological conditions during clinical trials (Mehra M.R. et al., 2020, Khan A. et al., 2020, Brass E.P. and Hiatt W.R., 2006). The key benefit of the work under this licence is to be able to eliminate substances, early in their development, that induce unacceptable toxic side-effects in pre-clinical species before they are used in humans.

Short-term benefits, achieved within the life of the project, will include the provision of a package of high quality and robust pre-clinical data that will facilitate the rapid identification and selection of the best candidate drugs and avoid any unnecessary and wasteful *in vivo* tests being applied to inappropriate drug candidates. The main purpose of the project is to identify the No Adverse Effect Level dose, which will advise an optimum safe dose for “first-in-man” studies, this will not only help protect humans but will also safeguard funds which can be used to advance other substances of interest, thus maintaining study integrity. Data generated from acute toxicokinetic studies will also be useful in indicating appropriate drug doses and concentrations used *in vivo* for chronic toxicology assessment, thus maximally reducing the levels of animal potential suffering. The immediate benefit would be the knowledge to inform further drug development. Maintenance of a test substance’s research path continuity will be achieved in some cases, as some substances will be already explored by us during *in vitro* and PK programmes, therefore producing more harmonised data which can potentially lead to animal use reduction due to better understanding of chemical/physical/pharmaceutical properties of drug candidates and improving statistical significance of the data obtained. Additionally, financial income from the research work will allow Company to re-invest in facilities, the most current equipment, training of staff in new relevant techniques to improve delivery of 3Rs.

For the mid-term benefits, achieved within 2-7 years, studies performed under the authority of this licence will allow identification of drug candidate’s suitability for further development towards use in human trials prior to administration to clinical patients.

Long-term benefits will be achieved mostly at the human trials stage and beyond and will include the identification of the dose level and duration of treatment that is likely to compromise optimum efficacy of effective substances in human trials and clinic. Other long-term benefits lie outside the direct control of this project licence, however, ultimately, this project is expected to contribute to the treatment and prevention of disease in the clinical field through screening out harmful drug candidates and allowing only effective non-toxic treatments to be further developed for the benefit of the patients.

Who or what will benefit from these outputs, and how?

It is expected as short-term benefits, a certain number of compounds would be identified as toxic due to the current programme of work and be prevented from going into further development and some compounds would be identified as potentially suitable for use in clinical trials, benefitting client pharmaceutical research

organisations.

Generated data will also assist regulatory authorities, such as MHRA, EMA, or FDA and other regulatory bodies controlling the issue of new drugs to the public, in the decision-making whether to allow the progress of substances for further human clinical trials. The data produced is likely to be submitted to support the dossier of information for a new compound or used to guide the studies required to obtain such information, thus benefitting the wider scientific and pharmaceutical community. The data produced will also be of benefit to other researchers in the field by better understanding of toxicological effects of novel drug candidates and avoiding unnecessary *in vivo* experiments with identified toxic compounds. Recommendations of the dose and schedule to be used in "first-in-man" clinical trials with the new drug candidates will also be made, benefitting both trial patients and clinicians. Elimination of toxic substances early in their development stage would allow us, our clients and, eventually, NHS, to save resources and development time not only for mid-term, but also in the long-term.

Importantly, the animal data generated will provide input in determining the benefit–risk ratio where the potential improvement in the health and welfare of a human patient is compared to the likely health risk to that patient, which will give clinicians crucial information for informed control during drug approval, prescription and treatment process. The likely outcome being that the lead-time for new therapies to reach patients will be optimised, giving greater potential to save lives, improve therapy, alleviate suffering and reduce the incidence of adverse effects experienced with existing therapies in the long run.

How will you look to maximise the outputs of this work?

We follow a proactive publishing policy as it is in the interest of the business to develop new methodologies and to refine pre-clinical research in order to make the organisation more attractive to potential clients. It is anticipated that even though there will be limited opportunities for publishing obtained scientific data that is part of confidential early stages in drug discovery, we will make every effort to partner with our clients to publish relevant data output whenever possible for the wider scientific community. Our participation in the grant-funded projects and collaborations with academia have however resulted in publications, and it expected that will continue in the future from work carried out under authority of this licence. In any case and whenever possible we will try to disseminate our knowledge. Our aim would be that from our service work we would look to present short communications or posters at dedicated meetings, for example highlighting any developments in methodologies related to the 3R’s.

Species and numbers of animals expected to be used

- Mice: 2700
- Rats: 3600
- Hamsters: 600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice, hamsters and rats, as species of the lowest acceptable order, are the research species models of choice in drug discovery and development due to their size and substantial amount of literature data already available. These models have been used and validated extensively, and have provided much of our knowledge to date in toxicology studies which we plan to utilise to generate the package of high quality, robust and incisive pre-clinical data that we aim to provide. The choice of animals are also dictated by the governmental regulatory bodies which demand that prior to first-in-man studies, toxicology data are provided at least in rodent animal species to ensure that the drug is suitable for human use.

We will continuously monitor the literature to implement the latest animal husbandry legislation and practices. Furthermore, we will minimise animal suffering by using the most advanced technologies where possible, like appropriate anaesthetics, pain relief and infection control.

Typically, what will be done to an animal used in your project?

The careful selection of test substances from laboratory studies will ensure that only non-toxic potential drugs with a positive profile to treat human diseases will be taken forward for use in animal research. The design of proposed experiments will be rigorously considered in order to ensure that a minimum of animals are used at all stages without compromising the integrity of the work. In most cases, small groups of animals will be dosed with the potential drugs at low volumes and concentrations. Dosing could be done in the same ways as humans are dosed in clinic, for example through feeding tablets or powders or via intravenous injections. Animals will be carefully observed after the dose to establish toxic side effects of the drug and whether drug had any negative effect on behaviour. If no unusual behaviour or signs of toxicity will be seen, animals will be dosed with increased concentrations of the drug, until toxic effects will become visible, at which stage experiment will be completed. Each animal will be subjected to only one experiment to reduce potential negative effects of the unknown potential drug substances. Experiments can last either for a short duration, e.g. from one day up to one week, if immediate effects of potential drugs need to be studied, or a longer duration, e.g. for months, if researches want to establish cumulative effects and long-term effects of the drugs in question. During experimental procedure small blood samples can be taken from an animal to establish whether drug is still present in the body, what organs of the body it accumulated in to provide therapeutic effects and whether it affected body in any negative way. At the end of experiment animals will be painlessly put down and organs will be collected to be studied under the microscope to see whether drug has affected tissues even if animal did not show any unusual behaviour or side effects during experiment.

What are the expected impacts and/or adverse effects for the animals during your project?

All procedures will be carried out by trained, experienced and competent members of staff, so no adverse effects are expected from handling animals and experimental procedures. It is envisaged that some test substances at high dose levels may cause discomfort, and those animals undergoing repeat dose regimens may experience adverse effects such as reduced activity, abnormal behaviour, reduced food or water intake resulting in weight loss due to the possibility of drug accumulation. Animals will be monitored frequently throughout the study period and if any animal displays signs of pain or discomfort, such as changes in feeding, drinking or movement, or if the general state of health deteriorates from normal, e.g. non-socialising, excessive aggression or unusual behaviour, the therapy and pain relief procedures will be provided for each animal. In rear cases when pathological condition cannot be alleviated by therapeutic measures, animals will be humanely and painlessly put down to prevent any further unnecessary suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The nature of research aimed at identifying toxic potential of novel drugs during an early drug discovery process anticipates that some substances at some dose levels will cause toxic side effects. As it is difficult to predict which drugs and at what stage of development will cause side effects, we can predict the moderate severity category may be experienced by some animals. In our work we will be using exclusively rodents as species of the lowest acceptable order for the regulatory toxicology type of work. As study animals will be typically subdivided into groups to be subjected to ascending dose levels of the test drugs, e.g. control/low/middle/high dose level groups, we can predict that only around a quarter of all animals will reach moderate severity during studies due to screening-out of toxic compounds and doses. Whenever possible, studies will be designed and implemented to achieve the lowest possible level of severity. One of such refinement methods is a staged dosing approach when a drug is given at the low dose level and study is progressed to the higher dose level only in the absence of significant adverse effects in the lower dose group. Our previous experiments suggest only around 20% of rodents used for our toxicological research have reached a moderate severity with the vast

majority of animals staying within the mild severity level.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is of paramount necessity to determine the behaviour and assure safety of novel drugs in living systems to minimise the fatal risks to human patients. It is also important to select the most appropriate dose rates and administration routes of novel drugs to achieve a maximum therapeutic effect to cure various diseases without causing any unwanted side effects. Whole body assessments as described in this licence are therefore required by statute by regulatory authorities before prospective drugs can be administered to humans.
Which non-animal alternatives did you consider for use in this project?

The use of available data on chemical analogues and known toxicological profiles of related drugs, computer modelling and laboratory studies with isolated human cells and tissues are normally used for initial toxicity assessment, this information is taken into account to either completely eliminate the need for an animal toxicological work (e.g. with known toxicants/irritants or non-tolerated compounds), or significantly reduce the number of animals or potential side effects of the tested drugs proposed for human use.
Why were they not suitable?

Whilst laboratory systems provide us with important data on the properties of novel drugs, it is imperative for these properties to be determined in the intact biological system as computers or cell and tissue cultures have a limited application to the whole body response to potential novel drugs. This is because such experiments can only generate data restricted to few specific types of cells or predictive models and cannot cover hundreds of different cell types and thousands of interaction pathways between neuronal, vascular and immune systems present in a living organism. Despite positive laboratory results, animal work is still needed to confirm that the drug will not produce the undesired effects that have not been previously observed in cell assays. As such, current laboratory systems lack the complexity of living organisms in determining possible negative outcomes of drug-organ and drug organism interactions and establishing the degree of harmful effects caused by a novel drug and cannot be used as an accurate predictive tool to fully replace animal studies.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

A typical 90-day regulatory study will require 120 animals. We anticipate that we can do 2 studies/year with mice and 4 studies/year with rats for the duration of the project. To ensure safety of animals on long-term regulatory studies, short term non-regulatory pilot dose selection studies will be performed, which could require around 300 mice, 240 rats or 120 hamsters per year.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

For experimental planning and implementation, we will aim to follow “Planning Research and Experimental Procedures on Animals: Recommendations for Excellence” (PREPARE) guidelines to ensure scientific validity of the work and reduction of wastage in animals, time and resources. In our aim to reduce the overall number of animals used for the duration of this licence, and as a good scientific principle, we always insist that there is a well-established scientific rationale for undertaking any animal toxicology work and that prior laboratory data supports such work. Regulatory studies will be carried out in line with approved industry gold standard OECD/ICH guidelines, e.g. OECD 408 Repeated Dose 90 Day Oral Toxicity Study in Rodents, where study design and number of animals required were subjected to stringent international peer-review process, validation and approval. Nonregulatory discovery toxicology work will be primarily based on models available for regulatory toxicology, however other scientific models or methods published in peer-reviewed scientific literature will be used. We will be using contemporary designs for toxicity testing where we can derive a maximum amount of information from a minimum number of animals. Study objectives include determination of the most important clinical signs attributable to high doses of the test substance, time of onset and remission of those signs. These objectives are achieved by means of a comprehensive schedule of animal observations following dosing. For bespoke discovery toxicology work statistical analysis will determine the minimum number of animals that can ensure scientifically-robust results - internal expertise and external statistician consultants are available. To reduce animal use in acute toxicity testing, studies that include more than one dose group will be normally dosed sequentially, with an interval of at least 24 hours between dosing of subsequent groups. This will allow the effects of the previous dose to be fully manifested and will allow selection of the subsequent dose to provide the highest probability of contributing more useful information. Long-term toxicology and carcinogenicity studies requested for the same substance could be combined to allow for animal reduction. NACWO/NVS/AWERB committee may also be involved at the study planning stage to advise on appropriate scientific approach for discovery toxicology projects.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

A small pilot study may be planned to validate the proposed study design, staged/staggered dosing approach in a study design will allow meeting scientific objectives whilst minimising the number of animals at risk of suffering. To avoid bias between experimental/control groups and to use optimal minimal number of animals for each study, we will ensure identical study conditions/environment for all animals, which will be strain/gender/age-matched and grouped randomly. Where possible, a single control group as a comparator for a number of test materials will be used. We will aim to use both males and females for all our study designs, however to minimise the number of animals used, the testing can be performed in only one sex if sex is not scientifically important. During study execution it can be evident that not all substances will be allowed to progress through all the steps of protocol due to toxicity, also representing a part reduction in the numbers of animals during drug development process.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Procedures will be carried out in species of choice (either mouse, rat or hamster), which will be agreed with the

client, depending on the properties of the potential novel drug, the scientific test and the knowledge of the drug effects. The overall premise of the license is that the most refined, most relevant and least invasive methods will be used for each study. We expect the majority of work carried out in models with a minimum burden on the animals (short duration of a study, minimal number of procedures). When the experimental question justifies additional burden to the animal (i.e. single housing or repeated drug administration) we will provide additional adequate monitoring and will aim to keep such a burden to minimum levels. The work carried out will be of a moderate severity, with severity reduced where possible.

Why can't you use animals that are less sentient?

The toxicology research is often carried out to enable first-in-man clinical work with an expectation of adequate translation of research findings into human trials. Therefore, the choice of animals is often dictated by the governmental bodies, and as such, the minimally sentient species required for regulatory toxicology work are rodents. As one of the primary experimental endpoints for toxicological research is animal's behaviour as a reflection of toxic effects of the novel drug, e.g. affecting central or peripheral nervous, respiratory or cardiovascular systems, terminally anaesthetised animals are not suitable for required clinical and functional observations to achieve experimental aims and objectives.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimise suffering and stress, animals will be acclimatised after transportation and may be familiarised with the environment, procedures and handling prior to studies. Novel drug structural knowledge could allow prediction of possible side effects of the related chemicals. The physicochemical properties (e.g. pH, solubility) of test novel drugs would be considered to prevent nonspecific adverse reactions. Cannulation could sometimes be a convenient alternative to frequent animal handling and repetitive injection schedules. Some dose routes, i.e. rectal, can be beneficial by allowing for the avoidance of "first-pass effect" hepatogastrointestinal drug metabolism, thus potentially reducing the required dose concentration due to improved bioavailability, reducing associated side effects, and reducing the use of more invasive routes of administration such as the intravenous route. Appropriate anaesthesia/analgesia will be used, and the advice of the NACWO/NVS will be sought when necessary. Blood sampling volumes would not exceed recommended levels. For acute toxicity studies animals will be frequently monitored, whereas for longer-term studies daily assessment would be employed to minimise the risk of severe side effects being reached, a schedule detailing limiting clinical signs and humane endpoints is in place. In the unlikely event of animals reaching a moribund condition, or expected to become moribund, or experiencing significant pain or distress, they will be humanely euthanised. We will minimise animal suffering by using the most advanced technologies where possible, and by using appropriate anaesthetics, pain relief and infection controls. In addition to the above specific refinement measures, all the work will be completed in the state of the art modern environment using monitored facilities utilising equipment fit for purpose. The use of the individually ventilated caging, surgical suites, the rooms dedicated to particular techniques and robust cleaning techniques will ensure further non-specific infection and cross-contamination control as a refinement of the experimental procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We are committed to follow Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines, allowing us to ensure experiments are planned and conducted in the most refined way, suitable for Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines implementation. Our research facility is already accredited to Office of Laboratory Animal Welfare (OLAW) and Good Laboratory Practice (GLP) standards, with the strict implementation of the European Union (EU) Directive 2010/63 on education, training, competence, housing, care and use of research animals with a comprehensive set of Standard Operating Procedures (SOPs) based on OECD/ICH recommendations.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our managerial and research personnel regularly attend relevant scientific meetings, workshops, networking

events and continuously monitor scientific literature. The company holds regular Animal Welfare and Ethical Review Body (AWERB) meetings to share the latest animal husbandry, scientific legislation and the best 3R practices. These advances are implemented effectively by research facility Management through HO/NVS/NACWO monitoring programmes and regular training sessions facilitated by the Named Training and Competency Officer (NTCO).

Home Office

NON-TECHNICAL SUMMARY

56. Experimental investigation of microplastic ingestion bias

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Microplastic, Behaviour, Ingestion, Health, Welfare

Animal types	Life stages
Zebra fish	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to investigate whether certain characteristics, including size, concentration, density and composition, alter the tendency of zebrafish to ingest microplastic. We will determine the anatomical position of microplastic accumulation in the body, and investigate whether feeding behaviour influences ingestion.

A retrospective assessment of these aims will be due by 25 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In recent years plastic items have been found accumulating in the guts of marine and freshwater animals due to the increasing quantities of plastic dispersed into the environment. Fish are especially susceptible to ingestion of microplastic because such particles can be mistaken for food, with fish that eat plankton being particularly affected. Many fish species are consumed by higher trophic groups and there is also potential for plastic to be concentrated in the food chain. This research will demonstrate which types of microplastic are most detrimental to fish, with the potential to help improve their welfare in the future.

What outputs do you think you will see at the end of this project?

1. This project will generate new information regarding microplastic ingestion by zebrafish, exploring the extent to which microplastic size, concentration, density and composition biases the likelihood of ingestion; whether microplastic becomes trapped in zebrafish tissues; and if not, what their residence time is in the gastrointestinal tract.

2. We will publish our findings in scientific journals. We will aim for top-tier open access peer-reviewed journals with a broad readership in order to disseminate our findings to as many people as possible. We will also share our findings with the general public via press releases and social media.

Who or what will benefit from these outputs, and how?

Scientists interested in fish welfare will benefit from a better understanding of the correlation between microplastic type and harm caused to fish health. This may help inform future policy regarding pollution of the oceans and the types of plastic to use in manufacture.

Members of the public are increasingly aware of the need to protect the environment, including reducing or removing plastic waste. High quality scientific data showing the impact of microplastics on fish health and welfare will further raise awareness of this important issue.

How will you look to maximise the outputs of this work?

We will maximise the outputs of this work through collaboration, publication and dissemination. This project represents the first step in a new collaboration. The results obtained here will form the basis of joint grant

applications. We will publish our findings in open access journals as described above. We will further disseminate our results though our lab Twitter accounts and press releases, approaches that we have used successfully before.

Species and numbers of animals expected to be used

- Zebra fish: 90

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult zebrafish are the ideal animals to use in these experiments. They are small, easy to maintain in the laboratory and we know a lot about their behaviour, which can be used to assess changes to health during the experiment. They are also a model for other fish , allowing the information here to be used to understand other species including wild fish.

Typically, what will be done to an animal used in your project?

Animals will be fed one of two types of microplastic (polypropylene and polyethylene terephthalate) on the first day of the experiment. We will compare two different concentrations and sizes. The animals will then eat their normal diet for the rest of the experiment. Animals will be sacrificed at three time points (48h, 168h and 336h) using a standard method. We will then collect their organs and assess both the amount of microplastic eaten and its accumulation in different tissues of the body.

What are the expected impacts and/or adverse effects for the animals during your project?

The experiments proposed here will be used to generate pilot data for a larger set of experiments. While we do not expect adverse impacts during the course of these experiments, there is a possibility that the fish's health is affected by microplastic ingestion. This could cause a change in behaviour, (such as a alteration of locomotion or feeding, freezing, a loss of balance or increased time spent at the surface of the tank water) or even mortality.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The changes to health caused by microplastic ingestion in this pilot study could be severe. Previous research suggests that microplastic ingestion can be lethal to fish meaning that their behaviour and welfare needs to be monitored very closely. If we do not observe such mortality here we will apply to amend the severity rating for these experiments before carrying out further research.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 25 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The research described in this proposal investigates microplastic ingestion by zebrafish. Since we will analyse behavioural preference for different types of microplastic, accumulation in organs and the transit time through the body it is impossible to use cell lines or organ cultures for this research. I have looked at the FRAME website and the NC3Rs website for possible replacement protocols but have not found suitable alternatives.

Which non-animal alternatives did you consider for use in this project?

We considered using organ cultures for this research.

Why were they not suitable?

Organ cultures are not suitable, because we will investigate the feeding behaviour for different types of plastic (e.g. that sinks or floats) and the accumulation of microplastic in many different organs of the body.

A retrospective assessment of replacement will be due by 25 May 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated these numbers based upon data collected during pilot experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In general, we use the NC3R's Experimental Design Assistant tool when designing experiments in our laboratory. This allows us to check the statistical analyses and number of animals to use.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies to refine the size and quantity of microplastic to use in our main experiment. We will collect data from multiple tissues in each animal, reducing the total number of animals needed overall.

A retrospective assessment of reduction will be due by 25 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We have chosen to use zebrafish for this research because of the combination of well-established behavioural protocols and ease of maintenance in the laboratory. Adult wild-type zebrafish (AB/AB strain) will be housed in the best possible conditions in our aquarium. This aquarium has constantly circulating water which is regularly monitored for quality. Fish are maintained at low stocking density in specially designed tanks.

Fish will be minimally handled during the project. Pilot experiments will be used to calculate the concentration and size of microplastic to use in our experiments. In the case of unexpected adverse effects caused by feeding plastic (e.g. a reduction of swimming, freezing, loss of balance or surfacing), the experiment will be terminated. The Named Veterinary Surgeon and Named Animal Care and Welfare Officer will be contacted for advice before feeding microplastic to fish again.

Why can’t you use animals that are less sentient?

The goal of this research is to characterise the fate of microplastic in fish following ingestion. Species that are less sentient or that have been terminally anaesthetised may not eat any microplastic, making it impossible to carry out these experiments.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will refine the procedures that we use by monitoring the behaviour of fish during the course of this research during routing health checks. We will look for signs of distress, including changes to swimming, freezing behaviour, or an increase in opercular beat rate or tail beat frequency, indicators of pain in zebrafish. In case of unexpected change to behaviour we will terminate the experiment and contact the Named Veterinary Surgeon and Named Animal Care and Welfare Officer for advice before carrying on.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the ARRIVE and PREPARE guidelines, both when designing our experiments and publishing our findings.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in the 3Rs by reading scientific literature as it is published and by monitoring the NC3Rs website. We will use this information to update the protocols that we use when possible.
A retrospective assessment of refinement will be due by 25 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

57. Experimental modelling for a richer understanding of the biology of dementias

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Central nervous system, Cognition, Dementia - Alzheimer, Proteinopathies, Novel therapies

Animal types	Life stages
Mice	neonate, juvenile, adult, aged
Rats	adult, juvenile, aged, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Diseases of the central nervous system, and dementias in particular, are amongst the most difficult to cure and have been on the rise given the ever more ageing population. The global ambition of this project is to translate the disease from the human to the animal (here mouse and rat) and re-create models of the disease states so that a richer understanding of the underlying mechanisms can be achieved.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is vital to develop better and more appropriate experimental animal models of these diseases if we wish to make headway in 1) understanding the mechanisms underlying the diseases, 2) gathering knowledge about the underpinnings of the progression and endpoints of the disorders, and the putative targets for remedial actions so that novel therapeutics can be developed and tested.

What outputs do you think you will see at the end of this project?

1. New information: We will achieve a much more detailed and physiological relevant understanding of the mechanisms underlying dementia.

2. Publications: As in previous years, we will endeavour to publish our work in high ranking journals.

3. Models: We will have developed new and more realistic animal models of dementia, have investigated them in terms of their physiological pharmacological, cognitive anomalies and explore the usefulness of new and existing drugs.

Who or what will benefit from these outputs, and how?

As this is a translational approach, we aim for benefit to human patients (both median and long-term). We will test the generalisability of our animal findings in the patients (for example through direct translation of the EEG from mouse to man). This can be readily exploited and, if connected with drug testing, reveal immediate read-outs as to the benefit of the potential treatment.

How will you look to maximise the outputs of this work?

We already collaborate with a many colleagues around the world, especially in techniques which we have little or no expertise in, or where equipment is too expensive for single use in our laboratory. Moreover, we publicise our work at many conferences each year to engage in scientific discussions about our experimental directions and interpretations of our novel results. We develop new tests when old ones seem to be outdated or not applicable for the question we are asking. This all is published, independent of positivity or negativity of results. In case of particularly interesting findings, we would instigate press releases.

Species and numbers of animals expected to be used

- Mice: 15400
- Rats: 3650

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

During this project we will continually strive to develop/use novel animal models that more truthfully mimic the diseases. Advantages of using mice for studying human ageing and disease models include that the genetic and physiological parameters can be easily manipulated and they can be generated in a short period of time.
We will utilise a multitude of GA models, mainly mouse, which have been developed specifically based on the underlying pathology of diseases. In term of neuronal disorders, our focus in on dementias (e.g. Alzheimer, Parkinson, Fronto-Temporal, Lewy body and vascular dementia) and we seek to determine a complete disease profile. Most of these animals behave normally in their day-to-day home cage behaviour and endophenotypes only appear in an age-dependent manner and are revealed through our sophisticated scientific equipment only. Many of these diseases (dementia) are age dependent and only appear in older subjects. Our GA lines seek to accelerate the onset of these phenotypes to between 6-12 months of age. Animals will be kept up to a maximum of 18 month of age. To determine the exact age of onset may include juvenile subjects as pre-symptomatic.
- We will also use rat models (normal and transgenic or knock-out) with the same aims. Advantages include the size of subjects when surgical procedures are concerned, and the genetic similarity to human, which is greater than for rodent. These cannot always outweigh the disadvantages including weight of subject (in case of drug substance required for administration), breeding cycle, space requirements when it comes to size of equipment, etc.

Typically, what will be done to an animal used in your project?

There are potentially 5 methods of interference.
1. Genetic: this relates to the introduction (most frequently human derived) or deletion of genetic material to generate GA lines that mimic characteristics thought to be hallmarks of the disease in question. We have many of those seat our disposition and subject to availability, our collaborators will create new ones when novel more precise genetic tools become available, or when novel information about genetics needs consideration. GA lines of mild or subthreshold severity relevant to the plan of work may be added subject to a University approval process involving the NVS, the NACWO and scientist(s) out with the group. Information on mouse strains lines are held in a separate document (GA Record/Passport) available within the establishment.

2. Behavioural: This constitutes a principle read-out for the diseases investigated here (mainly dementias). We have a great variety of tests available and are constantly developing new paradigms with higher sensitivity or more appropriate for the research questions. Behavioural Tests which do not increase the harms currently detailed under the licence and are of relevant to the plan of work may be added subject to a University approval process involving the NVS, the NACWO and scientist(s) out with the group followed by consultation with the Home Office Inspector. Information on the Behavioural Tests is held in a separate document (Behavioural Record) available within the establishment.

3. Metabolic: Many older people are sensitive to metabolic syndromes, and these can enhance the risk for dementia. We have analysed numerous GA dementia lines and found that there are often comorbidities for

metabolic syndrome (type 2 diabetes like). Consequently, we will explore the influence of dietary manipulations in GA models and their influence on behaviour.

4. Pharmacological: Approaches to determine mechanisms in vivo include the administration of substances to block or activate cellular molecules. This is highly relevant for the understanding of what is wrong with the mouse, and at the same time instructive when it comes to developing new medicines.

5. Physiological: Coincident with behavioural alterations must be a change in neuronal firing patterns. These can readily be monitored using non-invasive EEG in humans, but it requires surgical implants in rodents. These, however, provide powerful tools to determine how abnormal physiological signatures can occur, and when combine with behaviour and pharmacology, how they may be corrected.

These 5 processes may be used as stand-alone protocols but may also be combined (see Table 1) to develop a much richer and holistic understanding of 'what is wrong with my mouse'!

What are the expected impacts and/or adverse effects for the animals during your project?

Animals may experience some form of side effects when novel drugs / drug classes are explored but this will be unusual and short term. Animals will be closely monitored for example for weight loss
Pain experienced during electrode implantation and other types of surgery is transient and mitigated by analgesic regimes. It is usually related to weight loss, which again is transient and animals quickly recovers.
Behavioural anomalies are normally subtle in most GA lines and only revealed by specialist equipment.
But we also have included some higher impact GA lines for Rett syndrome, in which young animals (similar to humans) mentally retard within the first 4-8 weeks (equivalent to 18 months of a human) of life and are co-morbid for movement and breathing abnormalities. We make particular provision for these subjects and have enhanced monitoring and early interventions in place to ensure they do not suffer.
The impact of single housing might be stressful for some animals, especially when working with female mice. This may be less so for male mice as they are used to solitary environments. There are multiple arguments for single housing, especially when metabolic analyses are attempted. To record exact food intake during high fat diet and limit food intake during dietary restrictions, animals need to be housed individually. This is the only way to accurately record food intake (and water consumption). It has the added benefit that all animals (male or female) are on the same housing regime and this enables reproducibility of study design and reduce variance in observations. Being single housed would facilitate behavioural observations also connected with single housing and reduce any interim stress periods otherwise occurring when animals are separated and re-united and this circle is implemented several times. Again, data robustness is of concern here.
As for the length of single housing (in mice and rats), the following arguments apply: i) for dietary studies, we cannot at present estimate the onset of effect in sone GA dementia lines so have applied for a long-term regime of up to 12 months. The exact regimes will be specified in study plans and are likely to be shorter, especially when an early intervention at <3 months is attempted. This may be different when we assess the dementia phenotype first and explore the add-on of dietary interventions thereafter.
ii) electrophysiological studies, the time lines would be similarly long as we would attempt repeat recordings in home cages in 3-6 months intervals.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Severities range from mild or sub-threshold to moderate. Although many protocols are classified as of moderate severity, only a small proportion is affected to this degree and many more subjects do not present with any harm. For both rats and mice, we therefore expect an 80:20% split in terms of mild versus moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are the only option for the analysis of behavioural functions correlated with brain activation. Towards this end, we seek to identify disease relevant markers (relevant for dementia) in the behavioural domain. These will be correlated with physiological recordings of brain activity, but also of metabolic influences (blood glucose). Including pharmacological treatments and post-mortem ex vivo analyses provides an enormously powerful tool to determine the mechanisms underlying the disease and to identify novel drug targets and novel treatment options.

Which non-animal alternatives did you consider for use in this project?

There is no suitable replacement or alternative e.g. cell culture, computer model, organelle, nematode, insect etc to using the whole animal to study behavioural / physiological changes and related brain function. For this we have opted to work with rodents as their behavioural repertoire is close to human, GA variants are readily available and translation between patient and animal can be achieved.

Why were they not suitable?

We need to use the whole animal in order to understand how changes in brain activity relate to behavioural / cognitive traits observed in normal and impaired individuals following ageing or neurodegeneration and this is not possible using the alternatives listed above. Although our laboratory also conducts in vitro / ex vivo based activities using neuronal cell cultures and molecular and biochemical technologies, these preparations alone cannot determine the behavioural and mechanistic objectives of this project.
During this project we will continue to improve and refine our experimental techniques and where possible minimise the number of animals used.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

- The animal numbers used during the course of this project will be the minimal necessary to draw statistically robust conclusions. We typically perform power projection analyses based on our expertise (behavioural, physiological, pharmacological and GA models) whilst taking into account that variables including age (we typically work with 6 weeks to 18-month old subjects) sex, species and background strain can influence sample sizes. Alternative means of power calculation may be derived from literature.

- Keeping up to date with the latest developments i.e. monitoring of the literature and attending conferences will ensure that we don't duplicate or repeat procedures with animals where data has already been obtained. An exception to this being when validation of the study via replication of the data is necessary (confirmatory experiments). Our laboratory is currently involved an EU Horizon 2020 funded project to assess reproducibility in preclinical studies (both within laboratory and between laboratories) with an impact on the Reproducibility Network UK and the 3Rs. We draw valuable information from this network in terms of study design and data robustness.

- Given our considerable expertise with many of the tests proposed in this licence, we would typically estimate a group size between 12-14 subjects in young subjects with clear genotype/drug dependent differences of 30%. Otherwise, cohort sizes may need to be bigger and variance will need to be established for novel tests.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

- This project will employ within-subject experimental designs where possible allowing repeated testing of an individual animal in a longitudinal design either across different experimental conditions or at different stages of development/age. Longitudinal designs have been found to reduce animal numbers by at least 50% compared to cross-sectional between-subject study designs.

- Multi-disciplinary/multi-factorial approaches will be employed in order to maximise the information obtained from a minimum number of animals e.g. implementation of a test battery of different behavioural assays or combination of EEG recordings with behavioural assessment.

- At all stages of experimental design, we will follow ARRIVE 2.0 guidelines with experimental blinding and randomisation in order to avoid any bias. We are also seeking to implement a novel 'Data Quality System' as pioneered by our EU project partners).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

- Pilot studies involving smaller numbers of animals will be utilised when exploring novel procedures. The data generated from these initial tests would be subjected to a power analysis in order to inform the appropriate sample sizes for subsequent studies required.

- Utilisation of advanced and sensitive analysis tools (incl. video analysis software, Matlab routines etc.) can also reduce the number of animals required to make data more reliable. Previous experience suggests, that markers in disease models need to differ by >30% from control values to be useful for drug assessment. This high level of abnormal behaviour goes against our objective to determine early small symptoms in order to initiate early treatment. Consequently, late onset phenotypes may require greater cohort sizes to differentiate from controls (n=16-19 when young or n=24-30 when >12months of age). The development of novel analytical methods using computational approaches allows us to identify endpoints during the early stages of disease and lower the putative suffering / noisier data set of older subjects. These analyses may reduce cohort sizes by 20-30%.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

- During this project we will continually strive to develop/use novel animal models that more truthfully mimic the diseases. Advantages of using mice for studying human ageing and disease models include that the genetic and physiological parameters can be easily manipulated, and they can be generated in a short period of time.

- We will utilise a multitude of GA models, mainly mouse, which have been developed specifically based on the underlying pathology of diseases. In terms of neuronal disorders, our focus in on dementias (e.g. Alzheimer, Parkinson, Fronto-Temporal, Lewy body and vascular dementia) and we seek to determine a complete disease profile. Most of these animals are normal in their day-to-day home cage behaviour and symptoms only appear in an age-dependent manner and are revealed through our sophisticated scientific equipment.

- The use of inducers/repressors, that activate/inhibit the expression of certain transgenes is typically not harmful for the animals. In terms of repressors, the inhibition of gene expression has beneficial effects for the subject and frequently improves live span and health status.

- While behavioural testing (cognition, movement related dysfunction, emotional responding) is the principle read-out of this project, it is difficult to draw conclusions on the underlying mechanisms from these tests. We therefore use additional endpoints, such as pharmacological treatment and metabolic blood parameters as informative tools to instruct on the underlying mechanisms of the malfunctions.

- These methods will be used as singular protocols, but a more powerful analysis is provided through the use of multiple approaches in the same animal. These include surgical administration of extracts/synthetic proteins/viral expression cassettes and pathological spreading of toxic proteins in all but some control animals.

- We will also use rat models (normal and transgenic or knock-out) with the same aims. Advantages include the size of subjects when surgical procedures are concerned, and the genetic similarity to human, which is greater than for mice. These cannot always outweigh the disadvantages including weight of subject (in case of drug substance required for administration), breeding cycle, space requirements when it comes to size of equipment, etc.

Why can't you use animals that are less sentient?

There is no suitable replacement or alternative to using the whole animal to study behavioural changes and related brain function. We need to use the whole animal in order to understand how changes in brain activity relate to behavioural/cognitive phenotypes observed in normal and impaired individuals following ageing or neurodegeneration. Many of these diseases (dementia) is age dependent and only appears in aged animals. Our GA lines seek to accelerate the onset of these phenotypes to between 6-
12 months of age. To determine the exact age of onset may include juvenile subjects as presymptomatic.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

- Animals undergoing surgery that need to be single housed post-surgery will be single housed one week prior to the surgery in order to try and minimise the stress induced in the animals following surgery.

- Self-dosing of analgesia will be utilised in animals with analgesia given via drinking water, we have also implemented bespoke analgesia regimes depending on the strain of animals used (eg. TG4510) and our experience of using them for surgical techniques. This has involved the administration of analgesia via drinking water prior to surgery.

- Refinements utilised in behavioural testing procedures include the use of advanced video observation tools and novel computational analysis tools/software to facilitate a more sensitive and effective profiling of animals in behavioural tests.

- We have developed a series of standard operating procedures for all regulated procedures and behavioural tests to ensure high quality data that is consistent and standardised with all laboratory members trained in these procedures.

- All surgical procedures will be carried out using aseptic techniques and performed to the HO Minimum Standards for Aseptic Surgery and the LASA Guiding Principles for Preparing for and Undertaking Aseptic surgery. Peri-operative and anaesthetic care measures will be implemented

following consultation with the NVS. The invasive procedures we use including implantation minipump or electrodes have also been refined (and will continue to be refined).

- Ageing rodents will be assessed and observed using scoring systems and humane end points established by other researchers with experience of ageing colonies and postoperative care typically including the appropriate use of analgesics and fluid food rations to support recovery. Selection of behavioural testing will account for frailties in their state of health (for example reduced movement, sensory impairments like visual, auditory or olfactory).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

- We are currently trailing a new Data Management System on the conduct of studies, their design and analysis tools, specifics required when working with collaborators from industry or academia and so on.

- We also use Design assist amongst other software tools for power calculation etc. We routinely use Matlab for writing new code for analysis scripts.

- Also, we follow the guidelines provided by commercial breeders (CRL: Guidebook on mouse and rat colony management) for housing and breeding of our colonies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The team have signed up for updates of the NC3Rs (as well as Norecopa) and get information forwarded automatically. We are fully aware of the advances made, as they also affect our work in several funded projects.

Home Office

NON-TECHNICAL SUMMARY

58. Exploring novel strategies for cardioprotection

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cardiovascular disease, cardioprotection, disease models, cardiotoxicity, cardiorenal protection

Animal types	Life stages
Mice	juvenile, adult, pregnant, embryo, neonate, aged
Rats	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to identify new ways of protecting the heart, improve current therapies, and possibly re-purpose some of the treatments already available for other diseases, if there is an indication that these can help improve heart disease. These new strategies will be tested on appropriate preclinical animal models with the view to translating into patients with different degrees of heart disease and with co-existing complex diseases such as diabetes, obesity, chronic kidney disease (CKD) and cancer; and corresponding medications.
A retrospective assessment of these aims will be due by 05 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Ischaemic heart disease is associated with narrowing or blockage of one or more blood vessels supplying the heart muscles resulting in deterioration of heart pump function. This is the largest cause for mortality as per the global estimates for 2016 published by World Health Organization (WHO). Projections for year 2030 suggest that if measures for reducing the prevalence of this disease are not in place, it will continue to be the single largest cause for death globally.
Coronary artery disease, the most common type of heart disease, causes blockage of one or more blood vessels supplying blood to the heart muscles leading to heart attack. When this occurs, the heart does not get enough oxygen and a part of the heart muscle wall that is supplied by the affected blood vessel becomes damaged. A large number of cells in the affected area die and since new cells cannot replace the dead cells in the heart, the damaged area will not be able to function normally. This increases the workload on the rest of the heart, which will further worsen the heart function with time leading to what is called heart failure and eventually death. The first obvious step to reduce the extent of injury to the heart is to restore blood flow by opening the blocked vessels using drugs and surgery. However, this sudden return of blood flow (called reperfusion) is known to make the injury worse. In addition to finding ways to limiting such reperfusion injury, an additional approach to decrease the global heart disease burden would be to reduce the incidence of coronary artery disease itself.
Coronary artery disease results from the presence of several cardiovascular risk factors such as high blood cholesterol, high blood pressure, obesity, diabetes etc. Most of the heart therapies in use are based on treating these risk factors and also implementing life-style modifications. Despite improvement in treating these diseases, the number of patients with heart disease continue to rise globally. This suggests that there are other, yet unidentified, harmful effects of co-existing diseases that may point to additional ways of protecting the heart and improving outcomes in these patients. Recent clinical trials have confirmed that in order to get the maximum benefit, it is important to understand how the different organs in human body interact in a state of health and ill-health, which in turn seem to affect how the body responds to treatments including those for cardiac disease. In addition to the diseases, advanced age is a non-modifiable, untreatable factor that has significant effect on cardiac health and efficacy of treatments.
Limiting the pandemic of heart disease is extremely important not only to reduce the global burden of this disease on health services and healthcare costs, but also to improve outcomes in certain other disease states such as diabetes, CKD and cancer. For instance, a vast majority of patients with diabetes and CKD die of

cardiovascular complications. Patients with diabetes mellitus (DM) are 2-4 times more likely to develop cardiovascular disease, which is the main cause for death in ~65% of patients with DM. In 2010/11, diabetes cost NHS about £9.8 billion, of which around £3 billion was spent on heart disease and associated complications. With diabetes projected to become the 7th leading cause of death worldwide by 2030, improving efficacy of treatment/management of heart disease in diabetic patients is essential to reduce the healthcare expenses. Another factor that can increase the chances of heart problems in the clinical setting is treatment with anti-cancer drugs such as anthracyclines. These drugs are cardio-toxic – i.e., they can weaken the heart muscle within a span of as little as a couple of weeks to as much as 10 years after treatment. As many cancer patients are over the age of 50, the chances are fairly high that they already have heart disease when diagnosed with cancer; and the cancer treatments can make their hearts more vulnerable. Hence the provision of life-saving chemotherapy is restricted in cancer patients presenting with cardiac risk factors. It is also known that cancer survivors with no history of cardiac diseases may develop heart diseases and heart failure as a result of chemotherapy. For instance, a significant proportion of childhood cancer survivors who received these chemotherapy agents show the development of heart failure in a fairly young age.
It is also extremely important to note that, not only do other diseases and certain drugs used for cancer treatment increase the propensity to developing cardiac disease, but heart disease can also increase the incidence of other diseases. For example, acute kidney injury is a frequent complication of heart attack and can strongly affect the short-term and long-term survival in these patients. Therefore, in order to effectively prevent and treat cardiac disease in young and old patients with/without co-existing pathologies, it is extremely important to understand how effective current heart treatments are in different patient populations.
To obtain data with potential beneficial effect for large patient groups, pre-clinical research involving use of animals mimicking human diseases are essential – more importantly to ensure that the refinements and new strategies do not pose health risk contrary to being beneficial. The insights from these will help refine and personalise current treatments and if needed, devise new treatment modalities.

What outputs do you think you will see at the end of this project?

The project detailed in this licence outlines research intended to 1) improve knowledge of mechanisms of cardiac cell death caused by decreased blood flow and other causes of cardiac injury; leading to 2) identification of new therapeutic targets; 3) development of new methods/drugs based on these newly identified targets for protecting the heart; 4) testing these new methods and also refining available treatments in animals harbouring diseases similar to human patients (e.g. diabetes).
Additionally, our project will undertake studies aimed at re-purposing clinically approved non-cardiac medications for application to cardiac diseases and chemotherapy-induced cardiotoxicity, if there is an indication that these may help protect the heart.
The insights gained from the studies carried out under this project will help improve the ways by which heart can be protected in the larger patient population who may present with several other clinical conditions in addition to cardiac disease. The knowledge obtained from this research can also contribute to the scientific understanding of injury caused by both the lack of blood flow and subsequent return of blood flow by drug or surgical interventions, as occurs in other vital organs such as brain (stroke), kidney and liver, and during procedures such as cardiac surgery and transplantation. The new therapies may also provide valuable adjuncts to current clinical interventions.

Who or what will benefit from these outputs, and how?

Our work aims to improve current methods/drugs and to find new ways of reducing injury in patients who experience a heart attack. Since those who survive a heart attack frequently develop heart failure due to the damage to the heart, improving available treatment methods and developing new techniques will help patients to survive longer and have a better quality of life. A decrease in the occurrence of heart failure following heart attacks would also provide substantial cost benefits to the public health service. Using animal models that have disease conditions similar to humans (e.g., animal models with diabetes, chronic kidney disease or cancer models receiving anti-cancer drugs) we also plan to estimate the success of cardiac treatments in patients with

other diseases. This is particularly important since different diseases can increase the risk of developing heart disease and can change the way patients respond to treatments.
Short-term outputs from the project will be mostly in the form of new scientific information regarding how the cardiac disease progresses and new ways of targeting cardiac cell injury. These will be presented in the form of peer-reviewed scientific publications. Other research groups studying cardiac diseases and pharmaceutical companies developing therapies will be the immediate beneficiaries of these findings.
Medium-long term outputs expected of the project mainly include information obtained from studies using currently available therapies - where the refinement of current treatment methods is aimed for. The data from these will also be presented in scientific journals and may translate into modifications in clinical practice benefiting patients directly.
A decrease in cardiovascular morbidity and mortality in different disease settings can translate into substantial cost benefits to the NHS. Thus, the major beneficiaries in the long run are the healthcare network who can provide better care at reduced costs and most importantly, the patients who receive optimum personalised treatments positively impacting their quality of life.

How will you look to maximise the outputs of this work?

We will try to maximise outputs from our project by

1. planning the experiments such that maximum amount of data can be obtained while reducing number of animals used - e.g., using non-invasive techniques (such as echocardiography) to obtain important information on cardiac function as well as collecting tissue samples for biochemical analysis at the end of experiments

2. collaborating with other groups who carry out research in similar fields so as to optimise experimental protocols and avoid unnecessary repetition of experiments

3. collaborating with other groups who may be able to obtain data from the same animals - e.g., in experiments involving diabetic animals, other research groups interested in pancreatic cell function may be able to obtain tissue samples of interest in their research

4. publication of all important data (both positive and negative findings) from the research in peer reviewed scientific journals - regardless of the immediate impact on existing scientific knowledge in the field

Species and numbers of animals expected to be used

- Rats: ~5500
- Mice: ~5500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are planning to use rats and mice in our studies because;

- These species exhibit anatomical, physiological and genetic similarity to humans
  The small size, ease of maintenance, and short life cycle offers the advantage of studying the different
-

phases of disease processes within much shorter time span

- Heart attack can be induced and the resulting damage to heart muscle can be assessed with accuracy and ease

- Heart function can be maintained by perfusion with crystalloid buffers, allowing examination of factors that are altered within the heart in the absence of external influences

- Reduced collateral blood supply in the heart - reduces the variability in the results between animals and thereby reducing the number of animals used in each experiment
- These animals can be genetically modified to express diseases that are seen in humans (e.g., diabetes kidney disease). This will provide a good model to understand how these diseases affect heart and to improve the use of currently available heart medications in patients who often also suffer from such diseases.

- These animals can be genetically modified to express proteins that are shown to be relevant to the development of heart disease or relevant to rescuing the heart from injurious insults like heart attack. This will provide a good model to test new treatment targets leading way to new drug development.

In experiments, where the immediate effect of a treatment on heart muscle function and injury, young adult animals will be used and they will be used in experiments under anaesthesia and humanely killed while still under anaesthesia - the tissues from these animals will then be used to obtain relevant information.
In experiments where the long-term effects of treatments and/or other diseases are to be studied, young adult animals will be treated with different medications or disease-inducing factors; maintained for duration sufficient to observe the effect of these treatments and then used in experiments. These animals will be monitored at regular pre-specified intervals to ensure that the relevant experiments are carried out at the correct stage of disease and the animals do not suffer unduly.
Aged animals may be used in both the above types of experiments, as a way to understand how and why the aged heart responds differently to heart injury and treatments.
Animals with changes in protein expression or harbouring disease-causing mutations will be obtained from established commercial suppliers and if possible bred locally. These will be checked to ensure the presence of desirable gene changes at the time they are weaned as pups and then let grow normally until they are young adults or aged before being used in experiments as stated before.
The choice between mice and rats will be based on the desirability of gene mutations in the study design. The commercial availability of large number of transgenic mouse models with gene expression changes desirable for studies involving cardiovascular disease makes it a desirable species to work with. Despite this, the relative larger volume of blood and tissues from rats makes them ideal for experiments involving repeated blood sampling for circulating marker measurements. Also, the recent development in inducing gene expression changes in rats may help decide the species chosen for each study protocol.

Typically, what will be done to an animal used in your project?

Breeding protocols - Mice will be mated and maintained under ideal conditions of housing. The litter of pups obtained will be checked for presence of the gene mutation and the randomly assigned to experimental groups. In some cases, these mice may receive pre-treatment with drugs that can modify their response to subsequent heart attack.

Tissue studies - Part of our work involves collecting tissues from animals and analysing these for different markers of injury and drug. In these studies, the animals may or may not receive treatment with drugs before or during heart attack; with the corresponding effects observed in tissues collected. The animals will be deeply anaesthetised (terminal anaesthesia) before inducing heart attack for a specific duration and then collecting the tissues. These experiments are particularly important to see any early harmful effects of treatments with respect to how the heart function when subjected to the injurious insult. Only after a beneficial effect or absence of

harmful effect at this point is confirmed that the treatment can be tested in a long-term recovery model (as stated below). An additional advantage of this model is that a lot more information can be obtained from the different tissues we collect from each animal.
Whole animal studies - Once the early/immediate phase of injury has been studied, the findings will then need to be extended to the whole animal model which sustain heart attack, survives with some heart injury and then goes on to develop heart-failure. This is particularly important if we want to take any promising intervention to the next step of clinical application in humans. In these experiments, the animals may receive treatments before, during or after the heart attack procedure, which will be done under deep anaesthesia. The animals will be allowed to recover after the heart attack, the pain being managed using pain medications as in humans. The duration of these experiments will range from a few days after the heart attack procedure to up to 4 months. The duration will be decided based on the extend of complications of heart failure we need to see in order to meet the study objective. In some of these studies we will use animals harbouring diseases like diabetes and kidney disease. This is especially important since the human patients who sustain heart attack often present with many more disease and health concerns in addition to the heart disease.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals will be used in experiments deemed absolutely necessary, based on initial data from nonanimal models. In a few experiments animals may be treated for a defined period of time with drugs which are already being used in patients, or in preclinical/clinical trials. Hence the safety, dose, and the methods of treating animals with these drugs are well-documented. This will help design experiments taking into account possible non-desirable side effects and effective management of the same. Although most of the drugs used are not expected to cause adverse reactions at the concentrations intended, the animals will be closely monitored and necessary steps taken to ensure their well-being.
- The severity limit set for the different procedures ranges from ‘Non-recovery’ to ‘Severe’. All the animals will be observed regularly for food and water intake, general features of discomfort (e.g. starey (puffed-up) coat, hunched position, lethargy, reluctance to move, isolation from the group, self-harm, changed nesting behaviour) and weight loss (maximum 20%). If these symptoms develop, the animals will be humanely killed.

Experiments using animals in this project involves administration of drugs and monitoring their effects on body tissues. These can be broadly classified into two:
- Non recovery – experiments carried out under general anaesthesia; deep anaesthesia will be maintained throughout the duration of experiment followed by a final overdose of anaesthetic to kill the animal without waking it up from the experiment. This method is not expected to cause any suffering as the entire protocol is carried out under deep anaesthesia.

- Recovery – experiments carried out under general anaesthesia; deep anaesthesia will be maintained throughout the duration of experiment. Upon completion of the experiment, animals will be allowed to recover from anaesthesia. These animals will be under continuous observation until they become fully mobile and start to feed and drink; which normally happens in the first 3hrs after completion of procedure. In animals undergoing heart surgery, wherein the chest cavity is opened, surgery performed and the wounds closed surgically before recovering the animals from anaesthesia, effective pain relief by medication will be provided to ensure that the animals are not in pain while recovering from the procedure and in the days thereafter.

Further, we also undertake studies on animal models with diseases such as diabetes, kidney disease and chemotherapy-induced heart failure. In these animals, the severity is due to the disease severity - a factor we need to include in our experiments so as to make any new findings applicable to the wider human patient population.
The animals will be killed by humane methods after the experiments and tissues of interest collected for further studies, making maximum possible use of the animals. At all points in time either during maintenance of the animals or during experiments or afterwards all possible steps will be taken so that animals do not suffer unnecessarily. Where the pain/suffering is not transient (occurring within the duration of recovery as a result of

the procedure undergone) and cannot be resolved by pain-relief medications, humane methods of killing will be used to end suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice - **Mild** severity (~20% in breeding experiments, <25% in tissue studies or non-recovery experiments, ~50% in diabetic mice), **Moderate** severity (<5% in kidney disease models, ~5% in diabetic animals), **Severe** effects (~5% in diabetic animals, ~15% in recovery/whole animal models, ~10% in chemotherapy studies).
Rats - **Mild** severity (<25% in tissue studies or non-recovery experiments, ~50% in mutant diabetic rats), **Moderate** severity (<5% in kidney disease models, ~5% in diabetic animals), **Severe** effects (~5% in diabetic animals, ~15% in recovery/whole animal models, ~10% in chemotherapy studies).
All the remaining animals will fall under the category of **Non-recovery** (i.e, no procedure afflicting painful, distressing or harmful effects has been carried out in these). Additionally, the animals used only for breeding purposes will not be used for any other experiments and therefore categorised under **Subthreshold** severity category - unless unexpected suffering observed, in which case the animal will be treated to minimise suffering and severity category recorded appropriately.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 05 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project aims to help heart survive better after a heart attack and to treat heart disease in patients with other co-existing diseases. The overall aim is that the findings will help improve treatment options for all patients regardless of their general health status. Such personalised medicine will improve the quality of life in the long term in these patients. Our initial ‘proof of concept’ experiments will be carried out on experimental models of non-animal origin. These models include cell lines - cells available commercially and grown in the laboratory, and also cells and tissue samples from human volunteers. Based on this pilot data, experiments that show promising results will have to be confirmed in more complex biological systems similar to the human heart and whole body before the treatment can be tested in patients. Since these studies cannot be carried out in humans until more information on the safety and effectiveness of the treatment is available, experimental models of animal origin have to be used. Among animal-based work, we initially use the isolated heart and cell models (which are of lower severity), and also get relevant additional information from other tissues from each animal. This will help to increase the output from each animal used. The studies will then be expanded to the whole body and recovery models (higher severity) only if positive results are obtained in the initial animal experiments.

Which non-animal alternatives did you consider for use in this project?

In vitro non-animal models -

Includes single types of cells, co-culture systems, cardiac tissue slices (organotypic heart slices), microphysiological systems like organ-on-a-chip and organoids - maintained in culture. All of these can be specialised by using cells or tissues obtained from patients with specific disease type. These can be useful in replicating the disease phenotype, validate possible genetic differences while comparing tissues from groups of patients who are more susceptible to disease or adverse reaction to certain drugs compared to other patients. These properties make in vitro models involving cells and tissues from humans ideal in drug toxicity and safety testing and to a certain extent can also help understand the interaction between different cells within the same organ (e.g. organotypic heart slices) and between limited number of organs (human-on-a-chip, where different compartments containing cells from different organs are maintained).

Ex vivo tissues from humans -

Tissue biopsies can be obtained postmortem or from live human patients (with prior consent) during cardiac surgical procedures. These may be a good alternative to tissues obtained from animals subjected to interventions so that they develop disease phenotype.

Why were they not suitable?

In vitro models - We do use *in vitro* cell cultures, specifically immortalised cell lines from humans or animals in our preliminary experiments. We also use tissues from consenting patients whenever possible, however the usability of these tissues is greatly restricted by the short duration within which the experiments need to be carried out. These tissues are usually obtained from patients with heart diseases and due to lack of blood flow within the tissue after excision, the core of the tissue dies with time rendering it unusable in subsequent experiments that require prolonged incubation with test drugs.
While the cell and tissue biopsy models are ideal to test the 'proof of concept' or 'test the effect' studies, these do not replicate the complexity that is seen within the intact human heart - be it in terms of the structure, the function or how the cells react in the native tissue to injurious insults. In order to achieve this, we will need to test the treatments in intact organs. Although the organoids and organs-on-a-chip seem like an attractive alternative for this purpose, we are not able to use this owing to the following reasons:
Firstly, the technical expertise and facilities required to develop, establish and maintain these are beyond our reach and are prohibitively expensive to set up.
Secondly and more importantly, the premise of our project is to look at how the changes within the whole body affects the heart - in different disease conditions and in response to different treatments, over a period of time recapitulating the duration that it takes in human body.
The human-on-a-chip, at its current state is still limited by the absence of all the neuronal and blood borne factors that keep changing in response to the different bodily and environmental cues - for e.g., disease states, diet types, medications, physiological stress, gender differences, diurnal variations - to name just a few.

So, although we use *in vitro* models to obtain as much pilot data as possible and are an essential part of our overall research programme, we will need to rely on animal models when it comes to confirming our findings in the complicated biological system - that is the working heart complete with the circulatory and nervous systems that conveys signals between the heart and the rest of the body.
A retrospective assessment of replacement will be due by 05 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animals will be assigned to experimental groups randomly, as appropriate and necessary, and blinding of data prior to analysis will be done so as to prevent bias and ensure best research practice. Appropriate statistical tests will be used as applicable to the study design. The number of animals required in each experiment will be determined by statistical analysis - based on expected effect size of each output/data (pilot data), significance threshold set at 5% and at a statistical power of 85%. The statistical analysis used will be based on the number of factors studied in each animal (single factor, multi-factorial or randomized block designs). We use the

Experimental Design Assistant (EDA) available within the NC3R's, to design and arrive at the optimum number of animals required for more complex studies. Based on our previous work, we expect ~80-85% success rate considering all proposed protocols. This has been factored in while estimating the number of animals required for the different protocols. However, a considerable number of animals are expected to be common to one or more protocols. Hence, taking into account the number of different treatments we want to undertake in our research, the different experimental models that will be used in arriving at useful, reproducible, clinically relevant information, we have arrived at the number stated above for a project duration of 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Whenever possible, we undertake small scale pilot studies to estimate the size of change in the measured parameter(s) as an indicator of expected biological effect. If the effect is too minuscule to be of any clinical/biological relevance, then the study using that particular treatment modality will be discontinued. If the results are suggestive of clinical importance, but need further validation, then we plan studies involving animals. In this phase of planning and designing animal experiments, we use different guidelines/tools - For example, the (https://norecopa.no/media/7864/prepare_checklist_english.pdf); the 3Rs resources and the NC3R's Experimental Design Assistant (EDA). The PREPARE checklist provides us a framework to ensure we have closely considered all required factors including available literature in the field and the novelty of our own preliminary findings - so as to avoid unnecessary repetition of research and animal use. EDA helps design the study considering the different treatment groups, interacting/affecting factors and also suggests the number of animals required for obtaining robust, reproducible and statistically significant data. It also suggests the appropriate statistical tools that can be used for subsequent analysis of results.
In addition to the EDA, we plan to enlist support from the institute's statistics experts for calculating the number of animals prior to large-scale animal studies. For simple two group studies, we use online statistical tools or software like G*Power or ClinCalc.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To reduce animal use we will:

- Use alternative methods that do not rely on use of animals wherever possible - e.g. non-animal derived models in preliminary experiments.

- Using pilot data from non-animal studies to assess the need of extending the investigations to animal models.

- Plan experiments using minimum statistically relevant number of animals.

- Use of experimental techniques that will give maximum amount of data from least possible number of animals (e.g. using whole heart, aortic ring, blood and other tissues from same animal)
.
- Use viral vectors for mutagenesis – reduces (by at least half, if not more) the number of mice bred to produce transgenic strains
.
- Careful experimental planning to include the minimum required number of animals in experiments without compromising the quality of results - e.g. repeated cardiac imaging of animals to examine changes in heart structure and function with time
. This will reduce the number of animals needed to study all the time points and also will avoid variations that may be present between the animals.
- Regular monitoring of the experimental activity in the lab by the project licence holder and experienced senior scientists to ensure high quality of research and optimum use of animals.

A retrospective assessment of reduction will be due by 05 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats and mice will be used in this project as it is easy to measure extent of damage to the heart and experiments can be performed in a controlled manner
. Rats and mice with diseases similar to human conditions (for e.g. diabetes) will also be used in the project for studying heart disease in a setting similar to human diseases.
The study will use protocols where the animals are terminally anesthetised and tissues collected for further investigations. These methods will cause no pain, suffering, distress or lasting harm and will be the mildest amongst all the procedures in the project. In other experiments, animals will have to be subjected to cardiac surgery under anaesthesia and then recovered for a specific duration of project to study the effects of treatments on the heart. In these studies, unfortunately, occurrence of pain and suffering in the initial duration after surgery is unavoidable. To reduce the pain and suffering, the animals will be provided with pain medications at appropriate dosage and monitored regularly to ensure that there is pain relief. If these steps do not remedy the suffering, then advice will be sought from the Named Veterinary Surgeon on other possible measures, failing which the animals will be euthanized.
As refinement to techniques used during the study, we aim to

- assess cardiac function/infarction using minimally invasive techniques (MRI, Echocardiography)

- for studies involving genetically modified animals, we plan to use ways of inducing genetic changes that will greatly reduce the number of animals used (viral vectors for transgene induction)

- use drug-filled minipumps implanted under the skin for chronic drug administration. The process of implanting minipump itself does not cause any suffering, since this is done under anaesthesia and the region chosen for implantation is such that the process can be achieved with the smallest of openings on the skin and the implanted mini pump will not interfere with any regular activity

- use microsampling techniques (tail vein prick) for assessing diabetes in animal models. This will be done in animals fed normally, avoiding stress due to overnight fasting and also less harmful compared to cannulation of vein where larger volumes of blood may be lost

- use drugs for developing obese/non-obese diabetic models with different disease severities in a much shorter duration than genetic models; minimises suffering especially in studies addressing effect of diabetes and age on heart

Heart disease affects the long-term survival and quality of life in patients. To help provide better treatments for the varied patient population, it is important to have a better understanding of how the heart disease itself and heart's response to treatments is affected by different disease states. For this purpose, we will need to use animal models with diseases like diabetes followed by induction of heart attack. Unfortunately, no available non-

animal alternative is able to replicate all the complex responses that are seen in a whole animal model as this. We will ensure that the minimum necessary level of clinical symptoms are evident in these animals and not to the extent that it causes severe harm.

Why can't you use animals that are less sentient?

The development of cardiac disease has strong association with the aging process and the presence of cardiovascular risk factors such as high cholesterol. It has been shown that the incidence of these in juvenile/ adolescent or young humans is very less unless the disease is due to a genetic condition. It is also known that the hearts of young humans are more resilient to injurious insults compared to the adults and the aged. We find similar differences between cardiac cells prepared from neonate rats/mice versus that prepared from adult animals. The neonatal cells are different from the adult cells not only in their appearance, but also in their susceptibility to injury and general functional properties. Considering these and also the fact that our research is aimed at translation into the clinical setting where the vast majority of patients are adults with other disease states, we use mostly adult/aged animals in our studies. Where parallels can be drawn to clinical conditions in the young - for e.g. childhood survivors of cancer developing heart failure, we may use juvenile animals to mimic this disease of the young.

In order to keep the animal suffering to a minimum, wherever possible in our studies, we use animals that are terminally anaesthetised.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

- Refining experimental skills: In addition to the training undertaken for obtaining personal licence, new members of the research team will be trained, supervised and guided by more experienced investigators in all aspects of animal research including designing experiments, handling animals, recognising signs of pain, suffering, lasting harm and distress; and sacrificing animals by humane methods when necessary.
- Researchers will be assessed for competency before being allowed to carry out experiments on animals.

- Where animals are to be subjected to repeated experimental interventions (e.g. blood sampling at regular intervals in diabetic animals), the animals will be conditioned to the repeated handling by the same researcher or team of researchers - this will reduce the stress associated with the unfamiliarity towards the researchers.

- The researchers will be encouraged to adhere to the least stressful procedures for the animals, where possible. For example, microsampling of random blood glucose by tail vein prick will be carried out for routine glucose level checks as opposed to overnight fasting (for fasting glucose levels) and invasive blood vessel cannulation.

- At all instances special care will be taken to prevent and reduce animal suffering caused by the experiments. In experiments known to cause some degree of harm, the researcher will work closely with the animal care team ensuring that the general body condition and any signs of stress are recorded and remedial steps taken as soon as possible. To this end, a body condition score sheet will be made available on which all findings are recorded.
- We will work closely with the Named Veterinary Surgeon (NVS) and the Biological Services to help us refine our procedures and also for advice while planning new procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

<u>For general guidance on compliance to best practice in conducting and reporting research on animals:</u>

Guidance on the operation of the Animals (Scientific Procedures) Act 1986

Legislation for the protection of animals used for scientific purposes Directive 2010/63/EU as amended by Regulation (EU) 2019/1010
Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines
Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines

For choosing appropriate models of cardiovascular disease:

We will use the established models available in peer-reviewed publications, as appropriate to answer the questions we are trying to address - with refinements/ updates implemented if it supports the 3Rs. (E.g., Lindsey ML, Bolli R, Canty JM Jr, et al. Guidelines for experimental models of myocardial ischemia and infarction. Am J Physiol Heart Circ Physiol. 2018;314(4):H812–H838. doi:10.1152/ajpheart.00335.2017)

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in 3Rs by keeping track of refinements in techniques published in the literature and also by discussions with other research teams undertaking similar techniques and experiments. In our experience, we have found that involving the animal care technicians, the Institute's Animal Welfare Officers and the Named Veterinary Surgeon in the experimental planning phase is extremely helpful in implementing small but significant refinements to our procedures resulting in considerable improvement in animal experience. This may be as small a change as the bedding material to as significant as changing the route of drug administration and improving post-treatment monitoring. Additionally, we will make use of information available in resources such as NC3R's website to identify useful modifications with the view of refining and replacing animal use in research.
A retrospective assessment of refinement will be due by 05 April 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

59. Extracellular matrix mediated control of immune cell recruitment and positioning in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Leukocyte, Migration, Inflammation, Brain, Extracellular Matrix

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how extracellular matrix structures and inflammatory agents work together to regulate recruitment of immune cells.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Movement of immune cells from the blood to tissues is important in allowing us to fight infections by bacteria and viruses. However, movement of immune cells is also central to problems associated with a wide range of diseases including inflammatory arthritis, atherosclerosis and cancer.

The extracellular matrix is a network of factors (proteins and sugars) released by cells to provide scaffolds which provide structure to tissues. We are now beginning to understand that the extracellular matrix plays a more specific and dynamic role in biological processes beyond simply being a structural support.

Specifically the extracellular matrix lines blood vessels and forms a barrier called the glycocalyx. The glycocalyx is critical in controlling the ability of immune cells to leave the blood, a process that is vital in fighting infection. The role of this glycocalyx extracellular matrix barrier has been overlooked and a more complete understanding of this process will allow us to understand how immune cells are recruited during disease. Once we better understand this process we can develop drugs to treat and limit immune cell recruitment and help treat people with a wide range of diseases.

This project will define how these processes work during the body's normal response to inflammatory stimuli, e.g. infection. In the longer term these findings can be used to understand how these processes go wrong in disease, e.g. rheumatoid arthritis.

What outputs do you think you will see at the end of this project?

The main outputs of this project will be new information explaining how immune cells are recruited to fight infection and also during inflammatory based diseases.

In the longer term these insights will drive development of new ways to control immune cell recruitment and produce new drugs.

This knowledge will be critical to understanding and finding new ways to treat patients with autoimmune diseases, such as rheumatoid arthritis, and vascular diseases, such as atherosclerosis and cancer.

Who or what will benefit from these outputs, and how?

This project will investigate how the extracellular scaffold (glycocalyx) helps to control the immune system response to infection. Importantly, this includes the positive elements of the immune system allowing us to fight infections, but also diseases where the immune system goes wrong. The chemokine system controls the movement of immune cells from the blood to tissue, vital for fighting infections but also important in inflammatory based disease. We have previously failed to make drugs that target the chemokine system during inflammatory diseases. Inflammatory diseases remain a key hindrance to quality of life and health in the UK and globally.

This project will also produce findings that will help to improve the use of a new approach where the thickness of the extracellular matrix barrier (glycocalyx) is being used to diagnose disease and decide on the best treatment approach.

The same mechanisms of recruitment studied in this proposal also play a role in cancer cell metastasis and tumour survival.

How will you look to maximise the outputs of this work?

The data generated, both positive and negative, will mostly be suitable for sharing via publication or by being uploaded to publicly accessible data repositories.
Any outputs will be assigned a digital object identifier (DOI) to facilitate sharing and accessibility of them.
Outputs beyond traditional publications may include video demonstrations of our validated protocols that would be of use to the wider community.

Species and numbers of animals expected to be used

- Mice: 1700

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are chosen as they have been used in related approaches, paving the way for my research and providing vital details that will inform our design and understanding of this work.
Using mice for this proposal will enable comparison with existing knowledge and allow future development of drugs based on our work.

The life stages chosen will be to undertake experiments on mice at the adult stage of development. This will allow comparison to previous work which was done on adult mice.

Typically, what will be done to an animal used in your project?

In the first experimental approach animals with have an air pouch produced by injection of sterile air under the skin, while the animal is anaesthetised, on 3 separate occasions, each 48 hours apart to allow recovery. Reagents will be injected into the air pouch to induce and/or inhibit immune cell recruitment and the animal will be humanely killed.
In the second experimental approach animals will undergo surgery to implant a glass window over the skull, before injection of reagents to promote and/or reduce immune cell recruitment and to label the endothelial glycocalyx and immune cells.

What are the expected impacts and/or adverse effects for the animals during your project?

Air pouches are made while the animals are anaesthetised and they display minimal adverse effects. Injection of reagents to induce immune cell recruitment may cause low levels of inflammation over the next few hours, however, the mice do not display signs of discomfort.
Cranial window implantation, imaging and induction of immune cell recruitment are performed under anaesthesia, as a result animals have few associated adverse effects. Local bleeding lasting no more than minutes is possible during surgery to implant the cranial windows and weight loss (1-4 days) after surgery is also

possible, but the animals will recover quickly.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severity of breeding is mild.
The expected severities of the experiments are moderate and will consist of 100% of the animals involved.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Currently there are no good cellular models of the glycocalyx, partly because we do not have a good understanding of the glycocalyx structure in live animals. Thus, it is essential to do these measurements in live animals
It is not possible to replicate the complexities involved in immune cell recruitment using cell-based assays, therefore, these experiments must be undertaken in live animals.

Which non-animal alternatives did you consider for use in this project?

The first alternative to be considered are cell-based models of immune cell migration, including transwell and adhesion under flow systems. Transwell systems monitor the movement of purified immune cells in a plastic dish. Adhesion under flow systems analyse movement of immune cells in the presence of mechanical flow produced by a machine, this models the effect of blood flow.
We have used these approaches, in combination with biophysical experiments, to screen mediators of immune cell movement that can interact with components of the glycocalyx. These approaches have been used to screen for reagents that can interfere with these interactions and possibly inhibit immune cell recruitment and will be continually used to reduce the number of animals used.
Another consideration is the developing field of organoid cultures to recreate live animal conditions. These are simplified and miniaturised models of organs produced in vitro.

Why were they not suitable?

Following on from biophysics and *in vitro* cell models it is now vital to use *in vivo* approaches.
Neither of these systems (or organoids) can replicate the glycocalyx. We do not have a complete understanding of the glycocalyx structure at different parts of the blood vessel system, this must first be established to allow comparison for cell-based studies.
The complexity of immune cell recruitment cannot be recreated in cell-based experiments. The air pouch model and live cranial window imaging approach both allow analysis of the role of the glycocalyx in immune cell recruitment.

Our findings will help to try and inform development of better non-animal model systems for these processes.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

From analysis and consultation with a statistician and use of the National Centre for the Replacement, Refinement and Reduction of Animals in Research experimental design assistant ('NC3r's' EDA) has been used to calculate the number of animals needed:
Glycocalyx structure at rest and following treatment with mediators of immune cell recruitment using 30 individual experiments, each comparing a control and a treatment group.
Immune cell recruitment analysis, consisting of 20 experiments comparing a control and treatment group.
Analysis of glycocalyx content, consisting of 15 experiments.
Analysis of the effect of changing glycocalyx content (sugar chemistry) on immune cell recruitment, 3 different sugar chemistries and 3 different mediators of immune cell recruitment.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where possible multiple read-outs will be measured in the same experiment, e.g. glycocalyx structure before and after treatment with a reagent that produces immune cell recruitment.
The 'NC3r's' experimental design assistant has been used, and will continue to be used, to determine the minimum number of animals to provide sufficient power to analyse relevant effects sizes of treatments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Where necessary breeding will be designed to produce the number of animals needed and experiments will be undertaken on animals bred to optimise the number of animals used in this project.
Pilot studies will be undertaken when starting new experiments to inform experimental design, i.e. power calculations, again optimising animal use. Where possible these will be informed by cell-based analysis.
A continued effort will be made to share tissue from experimental animals. In particular it may be possible to undertake analysis of the glycocalyx from tissue from animals which have been humanely and also from tissues following systemic treatment with mediators of immune cell recruitment. Tissue from experiments analysing the glycocalyx in the brain blood vessel system may allow analysis of the glycocalyx in other tissues as well and inform future studies by ourselves and others.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques

during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The air pouch and cranial window approaches will be run separately but the data from both, in combination, will allow analysis of immune cell recruitment and the role of the glycocalyx using the minimum possible pain, suffering, distress or lasting harm to the animals involved.
The air pouch model will firstly be used to screen the numbers and types of immune cells recruited in response to different reagents and for compounds that may inhibit this process. This reduces the need for the more invasive cranial window approach, that involves surgery, thus acting as a refinement by using a less invasive experimental approach.
Implanting cranial windows involves surgery but is the only way to simultaneously directly image the glycocalyx and immune cell recruitment within a tissue. Thus the associated pain and suffering is the least possible to achieve this outcome.
Both involve moderate severity and direct glycocalyx analysis (via cranial windows) will be undertaken whilst the animal is anaesthetised. In the majority of cases mediators of immune cell recruitment, that may produce some inflammation in the animal, will be administered whilst the animal is under terminal anaesthesia.

Why can’t you use animals that are less sentient?

Our knowledge of the production and relevance of the glycocalyx is uncertain beyond its existence and importance to inflammation. For this reason analysis must begin in adult mice where we know the glycocalyx is formed, regulates immune cell recruitment and replicates effects seen in humans in health and disease.
Where possible glycocalyx imaging experiments will be undertaken during terminal anaesthesia. The only exceptions will be unavoidable as they will involve analysis of the glycocalyx over time and therefore the same animal must be analysed then allowed to recover before further anaesthesia and glycocalyx analysis.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Monitoring of all animals will be continually undertaken as described alongside all possible postoperative care and pain management.
Refined handling technique will be utilised throughout, all operators will be highly skilled and/or continually trained and updated with the latest refined approaches, e.g. handling.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All relevant NC3R's guidance and updates will be engaged with, including sign posting to published studies, e.g. FRY, D. 2014. Chapter 8 - Experimental Design: Reduction and Refinement in Studies Using Animals. In: TURNER, K. B. V. (ed.) Laboratory Animal Welfare. Boston: Academic Press.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Continued engagement with the NC3R's resources will be undertaken. In particular continued use of the experimental design assistant (NC3R's EDA) will facilitate regular assessment of the 3R's and recent developments whilst also enhancing experimental design.
Attendance of workshops organised by the animal facility will be undertaken as well as continued consultation with staff at the facility.

Home Office

NON-TECHNICAL SUMMARY

60. Extracellular regulation of insulin response and tissue function

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Diabetes, Insulin resistance, Obesity, Extracellular matrix, Glucose and lipid

Animal types	Life stages
Mice	embryo, juvenile, neonate, adult, pregnant, aged
Rats	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

Obesity and Type 2 diabetes manifest a defect in responding to the hormone insulin ("insulin resistance") and fail to maintain normal stable glucose levels in the blood. These conditions are among the major health problems affecting western and developing societies and are associated with significant impacts on individual well-being and economic cost to society. The aim of this project is to understand how insulin resistance develops and how we can target it to develop novel therapies for Type 2 diabetes and its associated metabolic conditions.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Conventionally, research into insulin resistance has mainly focused on studying the direct action of insulin on molecular pathways INSIDE the cells. Our recent work has shown that the proteins and molecules OUTSIDE the cells also undergo changes during the development of insulin resistance. Furthermore, when we removed one of these molecules in mice, we found that they had an ability to improve response to insulin, especially in the muscle and fat. This finding opens up an entirely new area of diabetes research and may prove more specific to diabetes than trying to target the inside of the cells.

What outputs do you think you will see at the end of this project?

Today, very few groups work in the area of studying the role of extracellular matrix remodelling in metabolic regulation. I believe that progress in this field will bring novel understanding of the pathogenesis of metabolic dysregulation and will generate a new paradigm involving the extracellular matrix signalling for hormone action and energy homeostasis. These efforts are motivated by public health demands as the prevalence of metabolic-associated diseases has increased to the point that it is in danger of overwhelming the financial and social resources of numerous countries and has devastating and long-lasting consequences for the quality of life of afflicted individuals. This epidemic of nutrient excess driving metabolic and cardiovascular disease will not be resolved without the development of new and more effective treatments.

Our newly funded research on the role of the extracellular matrix in cardiac insulin resistance and cardiac dysfunction will provide important novel insights into metabolic regulation and lead to new therapeutic opportunities for preventing heart failure in patients. Our studies utilising clinical and preclinical anti-fibrotic agents have huge translational potential. If proven promising, these studies will offer new strategies to improve health conditions of patients with diabetes and heart failure.
The final outputs of this project will include new information and publications.

Who or what will benefit from these outputs, and how?

Our study will provide significant benefits to other researchers in the field of obesity, insulin resistance, and metabolic disorders. The proposed study will generate a new paradigm for hormone action. Therefore, the results are highly relevant to all researchers with interest in hormonal regulation (e.g. leptin, glucagon, sex hormones, and steroids). I have a wide-ranging network in the scientific community of groups working on various aspects of metabolic diseases. These colleagues will learn about the results of the project through publications and meetings.

In addition to my primary questions on muscle insulin resistance, this project is relevant to understanding insulin resistance in general as data from other tissues that are important for glucose homeostasis including adipose tissue, liver, and brain will also be generated from the applied approaches. The generation of these data will benefit academics in other disciplines including neuroscience (behaviour, neuro-degeneration), hepatology (liver disease), and cardiology (heart failure), as well as all chronic age-related diseases. The proposed research also employs a wide range of methods including the unique two-catheter protocol of insulin clamp, laser Doppler imaging in mice, and various genetic and pharmacological approaches. These techniques will catch attentions of researchers not only in the basic science, but basic clinical science and scientists in pharmaceutical companies. We will ensure all potential academic beneficiaries are aware of the findings from the study by publishing them in appropriate broad interest, high impact scientific journals, and by presenting the results at national and international scientific conferences.

How will you look to maximise the outputs of this work?

For the academic audience of the project, the results of the research will be published in high quality peer-reviewed journals and presented at scientific meetings. The research findings will also be disseminated to the general public via outreach activities. For example, research results will be available to the members of the general public through public presentations, media, e-Newsletters, and multimedia releases. The outreach activities will be achieved through a specialist public engagement and innovation service at the establishment. We will also engage with contacts in the pharmaceutical industry through meetings and invited talks. We have strong pharmaceutical industry links with internationally respected partners, with which translating our research into practice will be greatly facilitated. I have extensive experience in oral presentations at conferences and have previous experience in working with industrial companies, which will enable the full success of my communication plan.

Species and numbers of animals expected to be used

- Mice: 5000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Genetically altered mice and mice that are treated with substances known or suspected to alter glucose metabolism will be studied in this proposal. Approximately 3000 mice will be bred, and 2000 might undergo further regulated procedures, over 5 years. The proposed research will provide significant benefits to other researchers in the field of obesity, insulin resistance, and metabolic disorders. The proposed study may generate new insights into how hormones like insulin actually work. These might, in future, lead to new and effective treatments for diabetes and its related metabolic disorders.

Typically, what will be done to an animal used in your project?

Animals may be on a special diet or receive drug treatment to intervene metabolic regulation and energy balance. For the measurements of metabolism and cardiovascular function, animals may undergo surgical procedures for the access of major blood vessels or the left ventricle of the heart. Animals are usually put on a special diet at 6-7 week old and the final metabolic measurements are performed at 23 week old and followed by a humane end point.

What are the expected impacts and/or adverse effects for the animals during your project?

The genetic alterations themselves are not expected to cause adverse effects but may influence whether the mice become diabetic (e.g., after being fed a high-fat diet). Diabetes in mice is often first suspected when the animals' water intake (and output of urine) increases. It can then be confirmed by a blood test. Close observation of the clinical condition and body weight will then ensure that animals are killed humanely while these deviations from normal welfare are still moderate. Tissues will be collected post mortem for further laboratory analysis.

Survival surgeries (e.g. vascular cannulation and implantation of minipump and telemeters) may cause pain and weight loss. Animals will be continuously monitored post-operatively and have their weight monitored at least daily. Analgesia will be administered as advised by the NVS. These adverse effects are expected to be temporary and their weight returns to pre-operative weight within a week.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The breeding and maintenance of genetically altered mice are expected to cause no pain, suffering or distress (Sub-threshold). A small proportion of these mice (<5%) may develop clinical signs of diabetes (Mild to Moderate). Survival surgical implantation of vascular catheters may cause a short term moderate pain (Moderate). Close observation of the clinical condition and body weight will ensure that animals are killed humanely when deviations from normal welfare are not improved with remedial. The estimated proportion of severities are: 40% Sub-threshold, 40% Mild, and 20% Moderate.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The mechanisms by which animals (including humans) regulate their body weight, glucose levels and fuel storage/energy expenditure are complex and involve the interplay of multiple organs (e.g. muscle, liver and fat) and pathways. These cannot easily be mimicked in vitro. For example, an antiobesity/anti-diabetic effect can really only be assessed properly by examining intact animals. The mouse is our best alternative species currently available for genetic manipulation and for the analysis of metabolism and metabolic pathways that mirror that observed in humans (particularly in relation to diabetes and obesity).

Which non-animal alternatives did you consider for use in this project?

In vitro cell culture systems (e.g. C2C12 mouse myoblast cell line, 3T3-L1 adipocyte cell line, and H9C2 rat myoblastic cells) have been considered and will be used to further understand the signalling pathways of a gene product which has been shown to play a significant role in glucose homeostasis in vivo. Moreover, computer simulations will be applied for mimicking functions of physiology or for the purpose of training protocols when available.

Why were they not suitable?

While these studies will provide insight into the basic cellular mechanisms behind glucose transport, extramyocellular factors involved in the control of glucose uptake (e.g. glucose delivery to muscle) are necessarily absent. Moreover, glucose uptake by isolated muscle preparations is extremely resistant to insulin (requiring suprapharmacological insulin levels) and contraction (requiring extremely high intensity contraction). It is likely that in some instances the rates of glucose uptake in vitro do not become high enough to test the glucose phosphorylation capacity of muscle.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This number is estimated based on the number of animals used in the current active project licence of the applicant and the trajectory of the research activity in the group in the next 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

During the experimental design phase, online tools (e.g., the NC3R's Experimental Design Assistant) and local advice will be used to inform the appropriate design.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Several methods will be applied to optimise the number of animals in the project.

- Pilot studies will be used to estimate variability and evaluate procedures and effects.
- Power calculation will be applied before and throughout the experiment to reduce the number of animals.
- Sharing of tissues between studies and projects will be considered whenever possible.
- Computer simulations will be applied for mimicking functions of physiology or for the purpose of training protocols when available.
- Efficient breeding and the use of quality animals and veterinary care will be applied to decrease variability, avoid unintended breeding and loss of animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Genetically manipulated mouse models combined with dietary intervention and pharmacological approaches will be used during this project. Adverse effects are not expected from the generation of these models and therefore these should not cause pain or stress.

Metabolic phenotyping methods including tolerance tests, calorimetry, vascular cannulation and the clamp experiments etc will be used for functional tests. Adverse effects are thoroughly considered and will be controlled and limited as explained in the earlier sections.

Why can’t you use animals that are less sentient?

The project aims to investigate mechanisms by which humans regulate their body weight, glucose levels and fuel storage/energy expenditure during adulthood. Therefore, animals at a more immature stage won't be suitable.

It is necessary to use mice for these studies because this species has been extensively used as a model organism in the understanding of human metabolic diseases. Importantly, gene targeting technology and dietary manipulation is widely available for the mouse and thus allows investigators to precisely establish casual relationships between genes and biologic processes. In order to determine the role of extracellular matrix remodelling on in vivo regulatory systems (e.g. muscle insulin sensitivity) associated with obesity, it is necessary to use mouse as the model species.

Terminally anaesthetised animals are not ideal for many of the non-invasive metabolic tests including tolerance tests and calorimetry tests. Moreover, it is widely understood that anaesthesia has a major impact on metabolic regulation, which will most likely cause more variation and make interpretation of data difficult.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

As aforementioned in the earlier sections, refinements include increased monitoring (e.g. immediate after the surgery, frequent checks will be made for the first 2 hours, then approximately every 4hrs including hours beyond 9am-5pm, or more frequently if so advised by the NVS, until condition stabilized), post-operative care (e.g. placing the animals on a heating pad during surgery and in a warming cabinet during recovery, until normal motility resumes), and pain management (e.g. analgesia will be administered as advised by the NVS). With regard to the infection, the use of antibiotics to treat apparent infections, or prophylactically in the case of any

immuno-compromised animals, will be as advised by the NVS, and training of animals (e.g. animals will be acclimatised for single cage housing prior to calorimetry studies, and animals will be acclimatised to the treadmill by initially placing mouse in the treadmill without the motor turned on followed by being habituated at walking pace (5m/min) for 10min) will be used to minimise the welfare costs for the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

ARRIVE guidance will be followed to ensure experiments are conducted in the most refined way. In addition, research publications in the area will be closely monitored and followed to ensure any advances in the techniques implemented.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Updates from the NC3Rs will be regularly checked by monthly newsletters. The Laboratory Animal Science Association (LASA) is another good resources for access to policy updates, support and guidance relevant to laboratory animal science, and regular sector news, training and events information through LASA’s newsletter and conferences.

Home Office

NON-TECHNICAL SUMMARY

61. Feed conversion efficiency and methane emissions in cattle and sheep

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
    - (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Feed efficiency, Methane emissions, Rumen microbiome, Ruminants, Methane mitigation

Animal types	Life stages
Cattle	adult, juvenile
Sheep	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall project aim is to increase feed conversion efficiency (FCE) and reduce the environmental impact from cattle and sheep by providing tools and an improved understanding of mechanisms to underpin the development of breeding programmes and novel (precision) management strategies.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Feed is the largest single cost in cattle rearing and lamb production systems, so reduced feed consumption (for the same amount of weight gain) will lead to increased farm profitability and reduced food costs. A reduction of £100 in feed costs for a finishing beef animal would represent up to a £10 million annual saving if there were 5% uptake across the approximately 2 million prime cattle slaughtered in the UK each year. In addition, ruminant production is under continued political and social pressure to reduce greenhouse gas (GHG) outputs, since cattle and sheep contribute to 66% of total UK agricultural emissions (BEIS, 2018). This project will contribute to government targets for reduction in UK emissions of greenhouse gases (net zero by 2045), through targeting direct reduction of methane emissions as well as through reduced feed use resulting from more efficient conversion of feed into products. This project will develop minimally intrusive alternative methods to estimate feed efficiency or methane emissions, and precision management strategies designed to optimise production efficiency and/or directly target methane output.

What outputs do you think you will see at the end of this project?

Outputs from the project will be:

- Data on feed conversion efficiency (FCE) and related/component traits for groups of animals of known genetic background.
- Rumen samples and genotypic information of a large set of animals including those showing extreme phenotypes for FCE.
- Understanding the relationships between FCE and methane emission traits and other animal traits (e.g. animal health, rumen microbial imbalance).
- Understanding diet effects (and novel feed additives) on FCE and methane emissions.
- Understanding the host genetic effects on the rumen microbial genes and communities by estimation of the genetic parameters.
- Cost-effective predictions of FCE, methane emissions, feed intake and growth rate using microbial genes and communities.
- Development of a functional microbial microarray including the most informative microbial biomarkers to predict the genetics of FCE and methane emissions as well as animals prone to rumen dysbiosis.
- Identification of networks and functional pathways of rumen microbial genes impacted by host genomic effects.
- Marker/proxy methods and low-cost instrumental approaches to estimate FCE and methane emissions. These could either be used in subsequent breeding programmes or form the basis of on-farm monitoring

tools to improve nutritional management of cattle and sheep.

- Data to support the development of precision livestock management tools that directly target the measurement, capture (and combustion) of methane from housed-ruminants.

Who or what will benefit from these outputs, and how?

The beneficiaries of this work will include academic scientists, animal breeding companies, farmers, governments, regional assemblies and other policy-makers, climate scientists, environmentalists, animal health and welfare groups and the general public.

Academic Impact:

- The research will reveal the host genomic impact on the rumen microbiome to an extent that has never been achieved before. It will provide considerable contributions to understanding of the host genome affecting the functional and genomic architecture of the rumen microbiome in bovines, with fundamental new knowledge that will also improve our understanding of the microbiome in other ruminant or monogastric species, including humans.

- The results of using rumen microbial gene abundances as selection criteria to genetically improve traits such as feed conversion efficiency (FCE) and methane emissions would provide a blueprint for any other trait potentially associated with rumen microbial information such as animal health and welfare traits or meat omega-3 fatty acid concentrations.

- The host genetic influence on the complex microbiome network and its association with performance traits will give new knowledge about the host genomic control of the rumen microbiome and its impact on performance traits and methane. Because most of the functions of the microbial genes are known, this will provide novel insight into the host genetic effects on rumen microbial functions.

- Using comparative functional genomics, the differences and similarities of the host genomic effect on the microbial genetic and functional architecture between species will be uncovered providing substantial new knowledge about the host genome - microbiome interactions across species. Of particular interest is the relevance to humans, e.g. research on the host genetic effects on the rumen microbiota and its association with body composition may give background information for human personalised medicine approaches to reduce obesity.

- This work will provide an understanding of the impact of novel feedstuffs (e.g. seaweed, oils) on methane output and feed efficiency in ruminants. It will also provide new knowledge on diet effects on abundances of the microbial community and microbial genes.

- The project will generate a database of key performance, efficiency and environmental metrics associated with housed cattle production linked to waste management, renewable energy and vertical farming. Knowledge will be gained on the development of integrated technologies and farming components which have extensive application for monitoring productive output and environmental impact of UK agriculture, with potential applications to further sectors.

The UK:

- Meeting the food demands of the growing world population, whilst reducing the effects of agriculture on climate change is one of the greatest challenges that agriculture has faced to date. This research project is expected to provide a unique animal breeding strategy, methane-reducing diets/additives and precision livestock farming solutions to address these challenges in order to achieve sustainable cattle production -

in particular considering that 39% of the global GHG emissions from livestock production are enteric methane emissions from ruminants. Methane is a highly potent greenhouse gas - a reduction of these emissions will help to meet the legallybinding national target of net zero emissions by 2045.

- Reduced production costs and improved efficiency will enhance the sustainability and reputation of UK beef, and ultimately increase the value of UK beef exports.

- Sustainable beef production will provide long-term stable rural employment, land economy and communities.

UK beef producers:

- Performance traits, such as FCE, vary substantially between cattle therefore genetic improvement is expected to have a substantial impact on the efficiency of using limited feed resources, as well as having a major financial impact - since feed is the largest variable cost of production.

- Based on the associations between the microbiome and phenotypes, algorithms will be developed to predict traits like FCE or methane emissions so that these traits can be predicted from the rumen microbial genes without the need for measurement of feed intake and growth rates. These measurements typically last at least 2 months, so there will be substantial financial savings for animal breeding.

- The reduction in methane emissions and utilisation of carbon dioxide from housed cattle will allow marketing of beef products and high value crops (such as leafy greens) as having a low environmental footprint. The climate impact per unit of beef produced will be reduced. This will provide a significant market advantage as the consumer demand for sustainably produced food with low environmental impact is increasing rapidly.

Consumers:

- The demand for food with low environmental impact is increasing, the development of a technological solution and aligned precision management tools (breeding strategies, technologies to optimise efficiency, low-methane feeds) will produce low carbon beef and crop products and will increase the acceptability of UK produce for local and foreign consumers.
  Animal welfare will be safeguarded.

Meat processors and supermarkets:

- Market research highlights the increasing consumer demand for more environmentally friendly produce, and reduced consumer confidence in meat-products has resulted in a reduction in red meat consumption in the UK. By sourcing animals from producers using the proposed developments, supermarkets will be able to market premium low-carbon produce, and increase consumer acceptability of UK red-meat.

Industry:

- Our extensive range of industry partners (breeding companies, feed providers, agricultural equipment manufacturers, technology developers) will ensure that outcomes of the research can be immediately implemented.

How will you look to maximise the outputs of this work?

Scientific outputs of the work will be disseminated through journal-publications, national/international agricultural conferences and through social media/dedicated web-pages. Results will be embedded into undergraduate degree programmes and doctoral-training programmes and leveraged in wider research activities (e.g. animal behaviour and welfare).
The work is supported by a large network of commercial industry partners linked to each objective. These

partners will support in disseminating project outputs and developed solutions and knowledge to the beef and sheep industry**.**
Dissemination of the work will be across the agricultural community and wider network of businesses connected to each collaborating industry partner. This will include trade events, farmer-workshops, press and online channels and the wider network connected to collaborating industry partners. **Species and numbers of animals expected to be used**

- Cattle: 4500
- Sheep: 300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using animals that are used in beef production, which includes juveniles and adult life-stages. Ruminant production systems are under significant and continued political and public pressures to reduce their environmental impact and optimise feed use efficiency. It is important to investigate methods of alleviating this impact using ruminants.

- It is not possible to develop new proxy tools for the measurement of methane and feed efficiency of livestock, or relevant breeding strategies without working with the relevant farm animal species under normal management conditions.

- Previous work has demonstrated the potential for modifying the diet of ruminants to mitigate methane and/or improve feed efficiency. There is also growing evidence that novel feedstuffs and feed additives can significantly reduce methane production. It is not possible to evaluate dietary effects on feed efficiency/methane output, without working with the relevant farm animal species under normal management conditions.

- Furthermore, the use of technologies and data-driven solutions for improving efficiency and reducing environmental impact of ruminant production systems are growing in importance. It is not possible to fully evaluate the use of technology and novel management systems designed for commercial application, without using farmed animals managed under commercial farming conditions.

Typically, what will be done to an animal used in your project?

Typically, animals in this project will be subject to experiments where feed efficiency and/or methane emissions will be measured using gold-standard techniques for measuring these traits. Feed efficiency is measured over a test period which typically lasts 56-60 days using feed testing stations (on both research and commercial environments) where electronic feed intake recorders are used. Methane emissions is measured using respiration chambers where animals are housed individually within the chambers for up to 4 days and gas emissions (methane and respiratory gases) measured.
Animals will be involved in one of the following: (i) studies aimed to develop commercially relevant breeding solutions for feed efficiency/methane emissions where genetic information will be obtained from blood and/or

rumen fluid samples; (ii) studies aimed to test commercially relevant diets and feed additives for reducing methane emissions where animals will be offered carefully formulated diets; or (iii) studies aimed to develop and use technology which directly mitigate methane emissions and/or improve feed efficiency - such as methane capture from environmentally controlled housing.

What are the expected impacts and/or adverse effects for the animals during your project?

We aim to ensure that animals perform well, according to their genetic potential, and all procedures in this project are mild. Dietary treatments are only likely to cause mild, transient digestive upsets. Sampling procedures (blood, faeces), methane measurements in respiration chambers, and recording of feed efficiency are minimally intrusive, whilst sampling of rumen fluid through naso-gastric tubes is non-surgical and causes only transient discomfort.
Animals will return to our or commercial herds and flocks, subject to veterinary certification and them not having received treatments that exclude them from the human food chain.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We anticipate that the severity of the procedures used in this project will be mild. Our aim is to mimic commercial farm practice as far as possible, and to develop solutions which are commercially applicable.
The most severe procedures used in this study is blood and rumen sampling where only short-term transient discomfort is anticipated.
It is not anticipated that animals will experience severe stress, fear or discomfort as a result of the procedures noted above.

What will happen to animals at the end of this project?

- Kept alive
- Rehomed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is not possible to evaluate between-animal variation in feed conversion efficiency and methane emissions, nor to test the use of new precision livestock farming tools for the monitoring and management of livestock without working with the relevant farm animal species under normal management conditions.

Which non-animal alternatives did you consider for use in this project?

Whilst we take appropriate opportunities to screen treatments that influence feed efficiency or methane emissions using *in vitro* systems, which may use either enzymes or rumen fluid, this work seeks to identify the animal genetic effects as well as interactions with animal management. It is not possible to evaluate between-animal variation

or novel markers, proxies or sensors to monitor animal feeding, performance, feed efficiency or methane emissions without using them with the relevant farm animal species under normal management conditions. It is not possible to assess the systems to capture and combust methane, without testing them on the relevant farm species and under commercial farming conditions.

Why were they not suitable?

These methods (lab-screening and pilot work) are helpful to refine and improve design, but to assess strategies for commercial application requires work to be conducted in farm animals, under commercial farm conditions.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For each component of the project, experimental designs and methods of analysis have been or will be discussed with a statistician. The exact numbers of animals required will vary with particular experimental designs and will be based on previous experience and the scientific literature.
For each experiment, an experimental protocol is submitted to the organisational Animal Experiments Committee which includes statisticians in its membership. The protocol includes: a statement of the objective(s) and a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material. Furthermore, protocols for each experiment including the above information and other matters such as methods of analysis are produced to conform to Institutional Research Quality Assurance requirements.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

For each component of the project, experimental designs and methods of analysis have been or will be discussed with expert statistical support. The design of individual experiments will maximize the information obtained from the minimum resource and will draw on the expertise of our collaborators and our own experience of running similar experiments to optimise the number of animals required for the project.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will take appropriate opportunities to screen treatments that influence feed efficiency or methane emissions using in vitro systems, which may use either enzymes or rumen fluid. Where required, pilot studies will be used to provide the baseline data to inform on larger trial work, and to refine sampling procedures and techniques for data capture. We will also use the opportunity to share data and samples with collaborators (internally and externally) to achieve maximum value from minimum resource.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain

management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

All measurements will be made on breeds of cattle and sheep and diets representative of major production systems in the UK. Methods to measure feed efficiency conform to International Committee for Animal Recording (ICAR) guidelines for recording to be used for animal breeding programmes. The respiration chamber methods of methane measurement is one of the few methods accepted by the Intergovernmental Panel on Climate Change (IPCC) for purposes of compiling national GHG emissions inventories. The chamber method is calibrated against reference standards and is necessary to validate less invasive, marker/proxy or high-throughput sensing methods. Until such methods are developed there are no alternatives to the chamber methods.

All sampling procedures are mild and designed to be minimally intrusive because we need animals to be performing according to their genetic potential and as per normal commercial practice.

Why can’t you use animals that are less sentient?

It is not possible to use immature, less sentient or terminally anaesthetised animals. The study aims to develop methane mitigation tools and methods of optimising efficiency that are commercially applicable to farming environments.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The procedures described here are already refined as a consequence of our work over the last 10 years and in use by our team. These include measurements of feed efficiency and methane emissions and sampling procedures for blood, faeces and rumen contents. Where we are developing new methods - i.e. capturing methane from a semi-sealed environment - we will implement pilot testing within the respiration chamber facility before up-scaling to a shed-sized system.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The feed efficiency methods conform to International Committee for Animal Recording (ICAR) guidelines for recording feed efficiency to be used for animal breeding programmes. The respiration chamber methods of methane measurement are the only methods accepted by the Intergovernmental Panel on Climate Change (IPCC) for purposes of compiling national GHG emissions inventories. Animal housing and husbandry will at least meet, and may exceed, that required under ASPA.
When publishing our research we will use the ARRIVE guidelines to ensure that we make all the relevant information known to others and help refine further studies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continually review our procedures throughout our work and seek less harmful alternatives whenever these are available. We will regularly visit the NC3Rs website to check for any new developments.

Home Office

NON-TECHNICAL SUMMARY

62. Functional Organization of the Midbrain Serotonin System

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

serotonin, dorsal raphe, neuronal development, mental illness, behavioural tests

Animal types	Life stages
Mice	adult, embryo, pregnant, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to understand how the midbrain serotonin system is organized to modulate psychiatrically relevant

behaviours (including depression, anxiety, post-traumatic stress disorders and etc.), how the organization of the serotonin system is determined during development, and how disruptions in the developmental programs alter this organization, possibly underlying diseases such as post-traumatic stress disorders, depression and anxiety.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This work will advance our understanding of the relationship between brain development and psychiatric disorders. This will enable the discovery of novel potential targets for the treatment of human neurological disease.

What outputs do you think you will see at the end of this project?

The principal benefits of this work will be the increase in our basic knowledge of how the nervous system works. We hope to better understand the relationship between the serotonin system and behaviours, including behaviours that are relevant in psychiatric disorders (including depression, anxiety, post-traumatic stress disorder, etc.). By way of context, psychiatric disorders are among the leading causes of disability globally with one in four people in the world affected by psychiatric disorders at some point in their life. In both the mouse and human brain, the dorsal raphe (DR) and median raphe (MR) are the source of serotonin to the forebrain and the most relevant serotonin neurons to psychiatric disorders. In this project, we shall address the outstanding question in the field, how is the DR and MR serotonin systems organized to modulate psychiatrically relevant behaviours? Second, we shall reveal how is the organization of the serotonin system determined during development. The answer to this question is the fundament of the third question, that is how do disruptions in these developmental programs alter this organization, possibly underlying pathology? The answers to these questions will advance our understanding of neuromodulation in health and aid the development of effective therapies for brain disorders such as anxiety and depression.
In addition, we anticipate making significant technical advances in the areas of imaging synaptic transmission events in acute brain slices and the technology of neuronal cell-type specific proteomics, which we hope will be taken up broadly by neuroscientists.
The intended outputs of the project, therefore, will be new knowledge as well as new reagents (viral vectors, genetically modified reporter mice) and technologies (tools and protocols for proteomic procedure and microscopy). They will be disseminated via the open access scientific literature and presentations, and also via the institute's public engagement team. Moreover, institute policy ensures free distribution of reagents, including mouse strains, after publication, and vector constructs are submitted to appropriate repositories for onward distribution.
Who or what will benefit from these outputs, and how?

Short-term and medium benefits will be new knowledge and methods, but longer term will be enhanced appreciation of the genes-to-cells-to-behaviour model for neuroscience, as well as the application of basic neuroscience knowledge to understanding mental disorder progression and providing a neuromodulatory context for the epidemiology of psychiatric diseases.

How will you look to maximise the outputs of this work?

Cross-labs collaboration is the signature of my institute and I will work closely with the neuroscientist in the department to disseminate any technical developments and knowledge. The culture of the local neuroscience society is very collaborative and there are tremendous opportunities for collaborating with a neuroscientist in the neighbouring institutes and universities. Building on our previous achievements, I have rich experience of collaborations around the world. I am confident that these collaborations will contribute significantly to maximize the outputs of this work.

The data, reagents, tools, protocols and software generated by this project will be disseminated via the scientific literature and presentations, and also via my institute's news media operation. For all objectives (including unsuccessful approaches), we communicate our data at scientific meetings
(conferences) and publish in peer-reviewed open access journals

Species and numbers of animals expected to be used

- Mice: 40000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Serotonin system in the brain is the most targeted neural system for treating mental disorders, including anxiety, depression, autism, PTSD, and so on. However, there are up to 43% of the patients stopped taking antidepressants due to the side effects [Carvalho et al.]. The side effects are caused by the non specificity of drug targeting, meaning the entire serotonin system is targeted but not just the subgroup of serotonin neurons that are actually responsible for the symptoms. To solve this problem, we need to understand which subgroup of serotonin neurons is related to what mental-disorder relevant behaviours, and what are the genes can be used to target these neurons specifically. By doing this, we can discover better therapeutic agents then would have better treatments for mental illnesses.
In order to achieve this, we have to use mouse models to screen for the neurons and genes that can be used as drug targets, and then record the activity of these neurons to confirm their function.
First, Mice are the least sentient animals that can be used for satisfactory tests of the roles of genes and neuronal pathways in mental illness relevant behaviours. There are depressed-like mouse models but not depressed-like fly models.
Second, the characteristics of serotonin system are highly conserved between mouse and human, and the data we obtained from mice are relevant to human.
Third, mice have readily modifiable genetic systems, including lines carrying genomically encoded modifications (recombinase drivers and conditional alleles). These are widely available from centres such as JAX and GENSAT. Furthermore, a large number of genetic tools have been developed and validated specifically for mice: AAV-, Rabies- and Lentivirus based viral vectors; fluorescent reporters and activity indicators; genetic manipulators such as CRISPR, optogenetics and chemogenetics.
Finally, the genetic and electronic/imaging technology now available ensures very refined experimental procedures and relatively large data-sets from fewer experimental animals.
All the behavioural tests are only for adult mice. Surgeries or drug administration may apply to younger stages because most mental disorders start to develop in young ages of the patients.

Typically, what will be done to an animal used in your project?

Perfusion fixation or Decapitation: All the animals undergoing either of the procedure will be euthanized under

anaesthesia by intraperitoneal injection (IP) of anaesthetic. Further procedures (e.g. decapitation or abdomen incision) will not be carried out until the anaesthetic depth is confirmed by using the toe pinch-response method to determine the depth of anaesthesia. After it is confirmed that the animal is unresponsive, perfusion fixation or decapitation will be carried out for brain tissue collection.

Non-surgical injections: Drugs like tamoxifen, 4-hydroxytamoxifen, clozapine-N-oxide, or their variants and etc., will be by administrated. Animals undergoing this procedure are not expected to exhibit any harmful phenotype.

Surgical procedures: Stereotaxic injection and surgical implantation in the mouse brain: During surgeries, we will typically make very small windows in the skull to gain access to the brain, deliver dyes or genetically modified virus. For some animals, we then will implant optical fibres or cannulas. After that, we will secure everything with dental cement prior to wound closure, if appropriate.
In utero electroporation: During the 30 mins surgeries, DNA will be injected into the embryos' brains followed by the delivery of very mild electric pulses. After electroporation, the embryos develop normally in utero and are born naturally.
All the behavioural paradigms used in this protocol are frequently used standard mouse tasks that are for models of mental disorders. All the tests used are acute tests and animals have a break in between tests to limits harms. There is no need for showing clinical signs in any of the steps. Animals will be observed closely during and after these tests to ensure they are physically capable to perform them and that no lasting harm occurs.
Behaviour tests in mazes and open field chambers: These behaviour tests include open field test, novelty suppressed feeding test, place preference test and elevated plus maze related mazes. In these behaviour tasks, the mouse is placed in either a new cage, an open field box, a maze or a chamber with lick-ports. The animals will move freely under a recording camera for 10–60 mins. The animals will be returned to the home cage after the test. These tests are expected to result in mild transient anxious feeling in the mouse.
Appetitive learning tasks: During the training session, in order to generate enough motivation for the animals to participate in the training, mice will undergo sustained food restriction for up to 2 months, maintaining 85% body weight. Mice have access to food during training trials. Right after the training session, when the bodyweight is still maintained at 85%, mice will be tested acutely in the assay. The animals will be returned to the home cage after the test. The tests do not result in more than mild discomfort during food restriction.
Sucrose preference test: One property of depressed patients is that they lack enjoyment of enjoyable activities. It has been found that mice can also develop this property. Mice always enjoy things that taste sweet under normal circumstances. In this assay, depressed-like mice enjoyed sweet (sucrose water) significantly less than normal mice. This test measures how much a mouse enjoys sucrose water compare to normal water. Before the test, mice are acutely deprived of water and food for 16hrs. This is essential to motivate the mice to drink from both the sucrose water bottle and the normal water bottle. During the test, the numbers and volume that they drink from each bottle are used for quantitative measurement reflecting their ability to enjoy sweet. The animals will be returned to the home cage after the test. The tests do not result in more than mild discomfort during food and water restriction.
Social behaviour tests: here we apply the social recognition, approach behaviours test and the tube test. In these tests, the test mouse will be placed with a second mouse and the interactions between the two are monitored. There will be no fighting between the two mice during these tests because of the design. The tests on this protocol are not expected to produce adverse effects any greater than transient discomfort, and no lasting harm.
Aggression test evaluates male aggression and social dominance. The assay allows two male mice to interact for 10 minutes. The procedure will be supervised for its entire duration. The mice may approach, sniff and chasing at each other. There may be a fight, however, we do not expect any injuries caused by the fight, and the session will be terminated early if open wound injuries are caused by an attack (very rare) or one single episode of attacking (biting) is longer than 5 seconds. Animals exhibiting any unexpected harmful phenotypes will be killed humanely. Animals will be killed humanely with 5 days after this test.
Aversive(fear) learning tasks:
Some of our experiments have to use aversive stimulation in order to model fear-inducing events, including the emotion associated learning and the memory of learned fear. These are key aspects of mental illness in which

strong emotional memory component is present, such as phobia, or posttraumatic stress disorder (PTSD). To study aversive learning, animals have to expose to stressors that mimic aversive experience happened to patients, and in this behavioural assay, we use mild electric shocks as the aversive stimulus. Animals will only experience one session (up to 20 mins) containing several mild electric shocks (up to 0.7mA) per lifetime. At least 60 seconds in between of each shock and no more 10 seconds of the total shock time. This is sufficient to allow us to study the psychological processes that underlie learning about stressful events. This is a well-established model for the fear learning component of PTSD and represents a refinement over some other models (e.g. underwater trauma). It allows us to precisely control the timing of cues predictive of an aversive outcome and the outcome itself (unlike more general stressors, such as exposure to the scent of predators) and because it engages the same brain circuitry as the mental health disorders that we are studying (unlike, for example, air puffs to the eye, which engages reflexive circuitry with has little relevance to our scientific questions), however, we will investigate further refinements to this procedure in parallel with our behavioural studies. Animals will be killed humanely with 8 days after this test.

What are the expected impacts and/or adverse effects for the animals during your project?

In terms of welfare costs, the mutations to be used in this programme are benign, the surgical procedures and associated kit are well established and so the needs for after-care monitoring and analgesia are well understood. It is critical to our scientific success that the mice behave normally because that is our measured dependent variable. It is therefore paramount in our design of studies and use of animals that we minimise all aspects of stress and discomfort.
Animals undergo non-surgical injections are not expected to exhibit any harmful phenotype.
Surgery: Stereotaxic injection, surgical implantation and in utero electroporation will be carried out aseptically. In all cases, animals will undergo one or two episodes of surgery under general anaesthesia. Peri- and post-operative analgesia will be provided; agents will be administered as agreed in advance with the NVS. Animals are expected to make a rapid and unremarkable recovery from the anaesthetic. They are expected to recover quickly and will be given painkillers and postoperative care. Animals are expected to reach the moderate level of severity exclusively during surgery and during the period immediately following the surgery, which represents <2% of the time spent by the animal. One day after surgery animals normally show no signs of discernible discomfort for the presence of implanted devices or as a result of injected tracers/virus. Any additional risk is readily mitigated by appropriate technique and after-surgery care.
After in utero electroporation, the embryos develop normally in utero and are born naturally. The dams will only undergo one episode of surgery and are not expected to show any signs of distress. In the uncommon event of post-operative complications, animals will be killed unless, in the opinion of the NVS, such complications can be remedied promptly and successfully using no more than minor intervention.
Non-aversive behaviour tests: These behaviour testing will be under standard protocols that will not cause any lasting harm and in most cases will cause only momentary mild discomfort or anxiety. Animals will be observed closely during and after these tests to ensure they are physically capable to perform them and that no lasting harm occurs.

Electric foot shock: Each mouse will only experience one foot-shock session (up to 20 mins) containing several mild electric shocks (up to 0.7mA) per lifetime. At least 60 seconds in between of each shock and no more 10 seconds of the total shock time. Although stressful for the animals, electric foot-shock yields very rapid learning and consequently the low shock intensities and short durations of shock used that tissue damage is never seen. Animals will experience pain during the shock.
Water and Food restriction: Animals undergoing food or/and water restriction are not deprived below 85% of their calculated free-feeding weight based on published growth curves of age-matched controls. On the rare occasion that an animal exceeds 15% weight loss compared to the published growth curves (with no other clinical signs) then it is given immediate access to additional food. Animals used in these assays are not expected to experience any long-term pain or distress. We will continue to monitor animals for any signs of distress. The tests do not result in more than mild discomfort during the withdrawal.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

85% of the mice are expected to undergo mild severity symptoms, i.e. breeding and mild behaviour tests. A further 15% will experience moderate severity i.e., surgery, electrical shock etc.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

My research seeks to understand how serotonin system modulates behaviours that are relevant to mental disorders such as post-traumatic stress disorders, depression and anxiety. These are products of an intact nervous system. It is the case that a large part of my work (ca. 60%) uses ex vivo brain tissues and so the requirement for animals is in suitable breeding programmes to generate intact tissues.
Beyond this, the use of conscious adult mice is unavoidable if we are to study the integrated circuitry and the dependent behavioural states and outputs: cell lines or computer simulations cannot yet come close to reproducing the complexity of the brain. Importantly, the starting point for our analysis of molecular mechanisms is our broad view of the existing literature of physiological and molecular biological studies of the serotonin system and much of this is derived from in vitro and cell-based studies.
Which non-animal alternatives did you consider for use in this project?

The starting point for our analysis of molecular mechanisms is our broad view of the existing literature of physiological and molecular biological studies of the serotonin system and much of this is derived from in vitro and cell-based studies.

Why were they not suitable?

Cell lines or computer simulations cannot yet come close to reproducing the complexity of the brain

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

In order to pursue the aforementioned objectives, we will need to make use of transgenic animals. We estimate that over the course of five years we will need to make use of about 30 distinct transgenic lines. These include mostly but not exclusively: population-specific CRE and FLP mouse lines, which will be used to identify genetically defined neuronal populations: various versions of reporter lines, which will be needed to visualize

those neuronal populations; activity modulators that will be crossed to FLP and CRE lines in order to alter the activity state of the identified neuronal populations. The establishment of those colonies will require about 3000 animals. Breeding and maintenance of the established lines is estimated to require, based on our recent experience, around 27000 animals for the duration of the licence. This is taking into account the need for refreshing colonies per year and expected usage of about 20 distinct mouse lines per year. We plan to maintain active colonies with 12 females and 6 males. These numbers ensure a smooth workflow for 4 lab members in order to provide access to the lines also in the case of parallel work with the same lines of multiple members (a situation that will occur frequently for the most commonly used lines, such as reporter lines). Six lines will be bred in the homozygous state and a further 14 lines will have multiple alleles which will give rise to high progeny but a low number of offspring with the correct genotype for experiments.
We estimate to use around 1000 animals for in utero electroporation, including wild type and transgenic animals. 500 animals in the protocol of administration of modifying agents will be used for activity dependent labelling of neurons. About 2000 mice will undergo behavioural testing without any surgical procedure. The use of approximately 1500 animals will only undergo surgery but not behavioural tests for tracing experiments, including 8 neural circuits. We estimate to use around 500 animals in the surgery and behavioural testing protocol for brain slice imaging and electrophysiological recording. 1000 animals in this protocol will undergo behavioural assays. 1000 out of 1500 animals in the aversive behaviour with or without surgery will undergo surgery. This is based on our goal to perform fibre photometry imaging (300 animals) and GRIN lens imaging (400 animals), given an expected statistically required sampling of 10 animals per recording site per mouse line. We expect to use 300 animals for the optogenetic manipulation for each protocol. We will use approximately about 1400 wild type animals as control and as the cage-mate/social partner in the behavioural assays.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where animals are necessary, we control breeding programmes tightly. Based on my 11+ years' experience of working with mice as the animal models, I will ensure the robust experimental design and generate statistically valid results from the minimum requirement of the experimental stock.
Dose ranges will be based on my prior experience and the literature. For initial work with newly acquired lines or viral vectors, small group pilot studies will be used to scope phenotypes. In order to use a statistically correct number of animals, we routinely perform power calculations to set the extension of our experimental cohorts. Furthermore, we take a number of steps to reduce variability in our procedures. The largest source of variability derives from the behavioural performance. To reduce this we take two steps: Firstly, we use isogenic lines so to reduce behavioural variability linked to genetic variability. By using inbred strains we shall ensure consistency of results, and minimise the variations between individuals, thus allowing us to keep the experimental cohorts relatively small. Regular breeding of core lines to a C57bl/6 background will ensure genomic integrity. Secondly, we will perform all the behavioural work at the same time of the day across different groups to avoid any potential circadian influence on the behavioural results.
Data generated by these studies will take the form of differences in behaviour between experimental and control animals; histological preparations showing the site of viral injections, localisation of electrode tracks or sites of labelled substances or reaction products; and electrophysiological firing patterns in different brain areas. Satisfactory results will be judged on the basis of comparisons between control and experimental groups using analysis of variance statistics.
The studies we intend to conduct will be designed so as to minimise the number of animals used to demonstrate a given statistically significant effect. Generally, we run pilot studies to explore the parameters of an effect before the final design is decided upon. Whenever a non-standard experimental design will be required, statistical advice will be sought.

Whenever possible we make use of ex vivo recordings, this will reduce the instances in which we have to perform in vivo acute or chronic recordings. which greatly decreases the number of animals used under these protocols. However, in most of these cases, we still rely upon a phase of in vivo work and rarely on behavioural training prior to the ex vivo work.
All publications are made consistent with ARRIVE guidelines.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Cryopreservation will be used to preserve important lines and remove the necessity to hold stock for extended periods. On occasion, we may need to ensure that our protocols are optimised and this may require the re-implantation of un-manipulated oocytes, embryos or blastocysts. Cryopreservation of embryos and sperm will be used for long-term storage of genetically altered mouse lines and pedigree lines with in vivo viability assessed to ensure that lines can be re-established successfully.
Rederivation will be undertaken should the health status of the animals be compromised in a way that would significantly affect the welfare of the animals or where the experimental results might be altered unduly.
Whenever possible we make use of human Embryonic Stem cell (hESCs) derived neurons in substitution for the in vivo work. We will collaborate with colleagues who devise neural organoid to develop neural circuit model for the serotonin system to reduce the number of animals.
We will significantly reduce our reliance on animal models by performing preliminary studies in cell lines. We will also employ, as far as possible, in silico and in vitro techniques, to minimise the use of intact animals as well as inform that use.
We hold ex vivo tissue in freezers available to use for molecular anatomical studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use mice as the animal models because 1) mice are the least sentient animals that can be used for satisfactory tests of the roles of genes and neuronal pathways in metal disorder relevant behaviours. There are depressed-like mouse models but not depressed-like fish or fly models; 2) the characteristics of the serotonin system are highly conserved between mouse and human. Investigation of the role serotonin system in mental disorder relevant behaviours in mice is therefore relevant to humans; 3) the mouse is the only animal for which transgenic strains are currently available that allow us to genetically identify serotonin populations that would be otherwise indistinguishable. This is crucial for the success of our project, as it relies on assigning individual function to identifiable neuronal populations.
The mutations to be used in this programme are benign. The surgical procedures and behavioural tests are well established and so the needs for after-care monitoring and analgesia are well understood. It is critical to our scientific success that the mice behave normally because that is our measured dependent variable. It is therefore paramount in our design of studies and use of animals that we minimise all aspects of stress and discomfort.
Why can’t you use animals that are less sentient?

In this protocol, we are going to address how genes and neurons in the serotonin systems control metal illness relevant behavioural phenotypes. By doing this, we can discover better therapeutic agents then would have better treatments for mental illnesses.
To achieve this, we have to use mouse models to screen for the neurons and genes that can be used as drug targets. We have to use adult mice for the behavioural tests because most of the mental illness behavioural phenotypes are most obvious and less variable during adulthood. We cannot use animals with a lower capacity to experience pain or distress because 1) mice are the least sentient animals that can be used for satisfactory tests of the roles of genes and neuronal pathways in metal disorder relevant behaviours. There are depressed-

like mouse models but not depressed-like fish models; 2) the characteristics of the serotonin system are highly conserved between mouse and human. Investigation of the role serotonin system in mental disorder relevant behaviours in mice is therefore relevant to humans. We cannot use animals that have been terminally anaesthetised because the two main outputs of this protocol are 1) behavioural phenotypes 2) changes of neuronal activity during behavioural tests. The mice have to be awake and freely moving to achieve these outputs.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The mutations to be used in this programme are benign, the surgical procedures and associated kit are well established and so the needs for after-care monitoring and analgesia are well understood. It is critical to our scientific success that the mice behave normally because that is our measured dependent variable. It is therefore paramount in our design of studies and use of animals that we minimise all aspects of stress and discomfort.
Many of our experiments require neural manipulations (brain surgery). The surgical approaches used are the least severe available, involving the smallest amount of tissue damage. Animals are given extensive post-operative care including analgesics. Animals are closely monitored throughout the experiments and any signs of problems with implants or other aspects of surgery are immediately dealt with, or, if this is not possible, the animal will be culled. Similarly, animals are closely observed and monitored during the recording experiments and interactions with other animals. All surgical procedures are carried out with the best possible level of asepsis and according to best practice (internal guidelines).
We routinely administer peri-operative analgesia, with scoring sheets to monitor animal welfare for a minimum of three days post-surgery. We routinely use non- steroidal anti-inflammatory drugs such as carprofen or meloxicam.
Some of our experiments necessitate the use of inescapable shock procedures in order to model aversive conditioning and fear learning. These emotional aspects are crucial components behind the mechanism of post-traumatic stress disorder (PTSD). We have to use foot shock as the fear-inducing events. For traditional fear conditioning test, we employed the most refined and well-accepted conditions, with 5 mild foot shocks (up to 0.7mA and 2s) in a session. Each animal will only experience one session (up to 10mins) per lifetime. For neuronal recording coupled with foot shocks, each mouse has to receive multiple foot shocks to minimise variables and ensure reproducibility. To ensure that we use the most refined conditions here to achieve the scientific goals, we carried out a pilot experiment in a collaborator's lab. We compared different shock numbers, duration time and foot-shock intensity and record the serotonin neurons' activity simultaneously. We found that, to generate statistically meaningful data for each animal, we have to use at least 12 shocks. Although the shock numbers increased compared to the traditional fear condition test, we also found that up to 0.6mA with 0.5s duration per shock is sufficient to evoke serotonin neurons' reaction. Taking together, after refining the protocol of behavioural test j, the total shock time an animal has to experience in one session is only 6 seconds, which is even shorter than in the traditional fear condition test. Moreover, conditions used in our ‘fear conditioning’ procedures are much more refined than the stressors employed by other behavioural paradigms, including forced-swimming, tail-suspension, learned helplessness, etc.
Overall, we are committed to refinement and minimization of animal suffering from ethical and scientific perspectives. As the outcomes of our experiments are primarily (often subtle) behavioural differences, a high standard of animal welfare is required for us to obtain meaningful data.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will follow the published Guidelines issued by LASA, NC3Rs, and RSPCA.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We continually refine our techniques as far as is reasonably practicable and we work closely with other PPL holders in the UK and across the world using similar techniques to share developments in best practice.

Home Office

NON-TECHNICAL SUMMARY

63.Gene and cell therapies for Ischaemic disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

pericytes, gene therapy, vascular grafts, angiogenesis

Animal types	Life stages
Mice	juvenile, adult, aged

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to find new cures for patients suffering from heart attacks and poor circulation in the legs.

A retrospective assessment of these aims will be due by 21 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cardiovascular disease caused by the narrowing of arteries and capillaries that provide oxygen and nutrients to the heart, brain, and legs, is the number one killer in the UK. Current treatments reduce the risk of death after a heart or a brain attack and delay the need of foot amputation as the last resource to save the life of patients with very poor circulation to the legs; however, many patients continue to have a poor quality of life. This is because current treatments are not enough to create new blood vessels around the occluded ones. In addition, grafts used by surgeons to create a new route around the blocked section of the artery tend to occlude after few years from implantation.

What is needed is a definitive solution for building up new blood vessels of different diameter, ideally large arteries to carry blood around the narrowed artery and capillaries to transport oxygen and nutrients to the suffering tissue. To reach this goal we will investigate new methods based on:

1) Testing drugs that can encourage the formation of new arteries and capillaries. For instance, using a protein which protects people who live a long life (centenarians) from suffering from blood vessel occlusion as they become old.

2) Producing artificial tubes containing human cells to create grafts like a real artery

What outputs do you think you will see at the end of this project?

In the medium term, 5 years from now, we expect that this research will demonstrate the proposed solutions are safe and capable of being done. We also expect to obtain new knowledge of how these treatments work. We will publish the results in medical journals where the acceptance for publication is warranted only after careful evaluation by expert reviewers. This will be a demonstration that the research is novel and accurate and has an important medical impact.

In the long term, the project aims to make these new cures available to the patients. This will require (1) preparation of documentation of main findings supporting efficacy and safety, (2) approval from agencies responsible for introduction of new medical treatments.

Who or what will benefit from these outputs, and how?

In the short/medium term, during the next 5 years, the work will generate new information about diseases that cause heart attack and poor circulation to vital organs in the body. We will pay particular attention to vascular cells that have not been investigated sufficiently in the past, such as pericytes, which are regenerative cells lining around the small and large vessels. One major goal is to incite the pericytes to act as building blocks to rebuild new and well functioning blood vessels. We will also work in the lab at producing new tube grafts containing pericytes and other vascular cells. We expect that, once implanted, these grafts containing human vascular cells will be superior to the ones used by surgeons to bypass blocked circulation.

In the long term, the work is expected to lead to clinical trials in patients. In due course, the work will be of direct benefit to patients and reduce the burden on the national health system and society caused by the disabling effects of vascular disease.

How will you look to maximise the outputs of this work?

The full demonstration that the proposed methods are valid and applicable to patients require skills and expertise that cannot be found in a single laboratory. We will therefore collaborate with other investigators, including doctors, surgeons, and scientists, to achieve the best results.

Clinicians are the best partners for maximising the impact of our research toward patients' benefit, but also to inspire new ideas from the bedside back to the bench. Scientists, with whom we collaborate, can bring new technologies and methods to the research.

We have established contacts with several small and large pharmaceutical companies (where some of our previous research fellows have relocated) and consult them frequently to see if they can be interested in helping us to develop our research and make faster the application to the clinic.

Results will be disseminated through scientific publications and presentations at national and international meetings. This is the most effective method to speed up the progress of science among the experts in the field. We will make sure that methods and results in publications contain enough details for other scientists to repeat the experiments and confirm or extend the acquired knowledge. The data will be communicated, either they confirm (positive results) or reject (negative results) the initial hypothesis. The report of data will follow the current international guidelines.

We have extensive experience in communicating the results through press release, having had articles covered in national news media (radio, television, and newspapers). The team is also experienced in communicating with lay persons through seminars to patients' forums and public lectures. We will use all the above methods to communicate results to the public and patients' associations after consultation with the Research and Development office.

Species and numbers of animals expected to be used

- Mice: 1350

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use mice, which are the mammals with the lowest neurophysiological sensitivity suitable for these studies. Wherever possible, we use the least severe model for our investigations. The disease we want to investigate occurs during adulthood and advanced age, which requires using adult and older models.

Typically, what will be done to an animal used in your project?

The animals will be subjected to surgical procedures to create the condition of blood flow blockade to the heart or limbs as occurs in patients. In other experiments, animals will not be operated but the natural evolution of vascular disease will be evaluated. Treatments will be given through local injections or systemic route. The duration of experiments will last from 2 weeks to 4-6 months, and we plan to use ~1350 animals during the 5 years of the project.

What are the expected impacts and/or adverse effects for the animals during your project?

Transitory pain after surgical induction of ischaemia will occur like that experienced by patients with a heart attack. Occasionally, we expect loss of weight and difficulty in breathing caused by heart failure and poor ambulation due to reduced blood flow to the lower extremities occurring for the maximum duration of 2 weeks.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

50% mild, 25% moderate, 25% severe.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 21 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We will be using mice for these studies because the complexity of cardiovascular disease and the efficacy of proposed treatments cannot be effectively tested in non-animal systems. However, preliminary studies of cell functions will be carried out before engaging with animal studies. In addition, simulation experiments are planned.

Which non-animal alternatives did you consider for use in this project?

Cellular models. Simulation of blood flow alterations using theoretical modelling.

Why were they not suitable?

Because the final demonstration of efficacy requires the complexity of a living organism.

A retrospective assessment of replacement will be due by 21 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Based on an average number of 30 animals per experiment for a total of ~45 experiments in 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Well designed and correctly analysed experiments can lead to a reduction in animal use whilst increasing the scientific validity of the results. To this aim, we constantly refer to the NC3R's Experimental Design Assistant as a guideline.

Here, we summarise the main steps considered in the experimental design to reduce the number of animals, control bias and ensure that the results are scientifically valid.

1) Pilot studies comprising a small number of animals to generate preliminary data and/or allow the procedures and techniques to be solidified and “perfected” before large-scale experimentation.

2) The minimum number of needed subjects will be calculated based on the expected average benefit and the expected variability of the benefit (assessed from the literature or pilot studies).

3) The allocation of animals to different groups of treatment will be at random: 1) to avoid biases, 2) to guarantee that groups have the same probability to receive a treatment, and 3) to control experimental variability. When planning attribution of animals to groups, the assignment of animals at random to different groups and sub-groups will be improved by dividing animals in blocks to achieve minimal variation.

4) We prefer to use genetically selected strains of mice because they show a more homogeneous response to the disease, meaning lower variability will allow a reduction in number of animals needed.

5) Important variables as sex, age and weight of the animal should be similar among the groups, again allowing reduction in variability.

6) Our facilities provide state of the art in environmental enrichment. We ensure that all the animals are exposed to the same enriched environment.

7) Collection of data will be done in a manner that the treated and control group have their measures collected at similar time and by the same investigator.

8) Biases will be avoided by ensuring that researchers analysing experimental outcomes are unaware of the treatment received (blinded) until the final statistical analysis.

9) Data will be treated according to the principle intention-to-treat, where all participants who are randomised are included in the statistical analysis and analysed according to the group they were originally assigned, regardless of what treatment (if any) they received

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We based our optimal number of animals and experiments based on experience in previous successful projects, pilot data and computer modelling. We also referred to the NC3R's Experimental Design Assistant to ensure that only the minimize number of animals are used.

We will use the best available technology and make sure that all the equipment is regularly calibrated for precise measurements.

A retrospective assessment of reduction will be due by 21 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are the less neurological developed species that can be used to test the therapeutic interventions we hope to progress into the clinical application. We will use the best measures and practices to attenuate pain, discomfort, infections, and stress. We will adopt refined microsurgical techniques to minimise the adverse effects of surgery. All animals will be carefully monitored after surgery and recorded individually. Wherever possible animals will be group housed and provided with enriched environment. Interventions to assess pain and suffering are refined to provide the maximum benefit for restoring wellbeing without interfering with the physiology of the animals. Therefore, observation frequency is calibrated to the risk of adverse events, which is highest during post-operative surgery. The definition of stress and pain severity is addressed using sensitive scales that have been elaborated for specific use in the specific protocol, for instance to identify low level of pain in mice with heart attack or occluded leg arteries. We also designed the protocol in a way the goal of the research is reached before animals reach the most severe outcome.

Why can’t you use animals that are less sentient?

Less sentient animals, e.g. fish or reptiles, cannot be used because do not reproduce the type of response a human being put in place following injury of the adult cardiovascular system.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have monitoring charts and post-operative charts in place which will be adjourned and improved as new evidence emerges from real experimentation or the available literature. Where appropriate, the animals will be trained to drug administration regimen associated with the lowest possible stress.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Surgical procedures will be undertaken in line with the recommendation set out in the LASA and institutional guidelines for aseptic surgery. Injection and drug administration will be conducted in line with NC3Rs recommendations.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep informed about advanced in 3R by reading the literature, attending courses and webinars. In addition, we will attend at least 1 meeting every year on animal welfare, such as the RSPCA/UFAW Rodent and Welfare Meeting or other 3Rs symposium.

A retrospective assessment of refinement will be due by 21 April 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

64. Gene silencing in early mouse development

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

X chromosome, Gene regulation, Development, RNA

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this work is to address a fundamental question in biology, how are genes regulated (switched on or off) during development from a single cell, the fertilized egg, to a complex organism made up of hundreds of different cell types. To tackle this question we study the process of X chromosome inactivation in female mammals, a highly informative model for understanding developmental gene regulation.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The proposed work will contribute to international research efforts aimed at understanding gene regulation during development. As we have now determined the entire DNA sequence (or genome) for many different animals, a central challenge is to understand how different genes, of which there are several tens of thousands, are regulated during embryonic development, in adult animals and in disease. Key findings that we make in our research contribute to our basic scientific knowledge, as described in textbooks, and this in turn is critical for the development of new applications, for example
in medicine and commerce. To disseminate our discoveries we publish our work in leading international peer reviewed scientific journals such as Nature, Cell and Science.

What outputs do you think you will see at the end of this project?

Through performing the studies under objective 1 we expect to make important discoveries towards understanding the role of a protein named SmcHD1 and its associated protein factors in X chromosome inactivation, and more widely in developmental gene regulation in mammals. Mutations in the human SMCHD1 gene are linked to the disease Facioscapulohumeral muscular dystrophy (FSHD) type II and our results should help to better understand the molecular basis of this disease and thereby to identify further areas of study. Our findings will be published in peer reviewed scientific journals and disseminated through presentations at international and national scientific conferences and at seminars in national/international research centres.
The X chromosome inactivation process is controlled by a ribonucleic acid (RNA) molecule, termed Xist, which accumulates over the entire inactive X chromosome to be. Our studies on Xist RNA localisation under objective 2 are aimed at understanding this process and will accordingly lead to discoveries concerning the fundamental mechanism of X chromosome inactivation. Our work will also inform our understanding of other examples of developmental gene regulation involving RNAs that localise to large chromosomal regions. Again, our findings will be published in peer reviewed scientific journals and disseminated through presentations at international and national scientific conferences and at seminars in national/international research centres.
X chromosome inactivation serves as a textbook model for understanding gene regulation and key discoveries that we make through the work described herein will be included in descriptions of the process used to teach students at undergraduate and postgraduate levels.

Who or what will benefit from these outputs, and how?

The impact of scientific publications resulting from this project will be realised in the short-term, influencing the

direction of research efforts in other labs around the world. Ensuing scientific advances will also have medium and long-term benefits in terms of providing scientific explanations in school and university textbooks. Discoveries may also be exploited in efforts to understand human diseases, notably FSHD type II.

How will you look to maximise the outputs of this work?

Collaborations with other colleagues/research groups offering different expertise/resources will play a central role in increasing the impact of published outputs. Dissemination will be through publication and presentations at conferences and seminars as above. Where possible negative findings and unsuccessful approaches will be publicised using newly established peer reviewed platforms such Wellcome Open Research and by depositing data in open repositories, Mendeley data, protein and relevant DNA/RNA/Protein databases.

Species and numbers of animals expected to be used

- Mice: 15700

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use the laboratory mouse to study the process of X chromosome inactivation that occurs during early embryo development. The laboratory mouse is the best studied and most widely used model for analysing embryonic development in mammals. Importantly, methods have been developed that allow specific genes to be added or removed in early mouse embryos, and these approaches allow us to investigate the molecular events that underpin X chromosome inactivation. For our work we analyse very early- through to mid-stage mouse embryos, the time window during which X chromosome inactivation occurs. Occasionally we use tissue from later stage embryos or adult animals.

Typically, what will be done to an animal used in your project?

The majority of mice produced under this licence (no more than 10,000) will be used for breeding to maintain strains with specific genetic characteristics and for embryo production. Up to 5% will be recorded as having experienced a mild procedure (those for which a second biopsy sample needs to be taken to determine their genetic type). All other animals will experience little or no effects from the breeding programme, referred to as sub-threshold.
A smaller number of animals will undergo surgical procedures (no more than 100 female mice will receive embryo transfers and no more than 100 male mice will undergo a vasectomy). These animals will undergo one procedure with full recovery expected without incident. The surgical procedures are needed to produce new genetically modified mouse lines.
No more than 500 female mice will be used for the production of early embryos using a hormonal injection procedure termed super-ovulation. This is performed to obtain embryos for experimentation or for long-term storage/archiving (cryopreservation).

What are the expected impacts and/or adverse effects for the animals during your project?

Superovulation procedures are expected to result in no more than transient discomfort and no lasting harm.

Surgical procedures will be carried out aseptically. In the unlikely event of post-operative complications, animals will be killed In the case of wound breakdown, uninfected wounds may be re-closed on one occasion within 48 hours of the initial surgery. Animals are expected to make a full recovery from the anaesthetic within two hours. The production or breeding of our mouse lines are not expected to produce a harmful phenotype in adult mice. Any transgene inducing agents normally have very little or no adverse effects on the overall welfare or health of the animals. In extremely rare cases, adverse effects may arise in the mother from the expression of genes in her foetuses, induced by the administration of substances. There is a very low risk of harm to the mothers from the method of delivery(<1%).
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Subthreshold 93%
Mild 1%
Moderate 6%

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

X inactivation is developmentally regulated and occurs only in mammals. For this reason our experiments are carried out using the laboratory mouse as a model organism.
In order to establish tissue culture models we need to first carry out a certain number of animal studies. For example to make tissue culture cells which have a particular genetic modification we must first make the genetic modification in mouse embryonic stem (ES) cells. The ES cells are then used to make a mouse with the modification on one of a pair of chromosomes. We then need to breed the mouse so that both chromosome copies carry the modified gene. Finally we make genetically modified tissue culture cells required for the study.

Which non-animal alternatives did you consider for use in this project?

Advances in genome engineering methods mean that some experiments can now be carried out in tissue culture models without recourse to animal experiments and we apply these strategies wherever possible.

Why were they not suitable?

In some cases tissue culture models are not sufficient to recapitulate the complex interplay of different cell types in a developing embryo and in these instances experiments need to be performed in live animals. Additionally, aspects of our tissue culture models are artificial and it is therefore important that we verify our conclusions in a truly physiological setting, mouse embryos.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Production and rederivation of genetically modified (GM) mouse lines will be carried out using standard methods and the estimate for numbers of animals used is based on previous work in labs performing this type of work, including our own. These numbers include allowance for the production of GM lines from independently derived embryonic stem cell lines as is standard practice in this field. The total numbers we have calculated for the duration of this project allow for the production of four novel GM lines, and the importation of two further GM lines obtained from colleagues or international mouse repositories.
Breeding of genetically modified animals will include matings to maintain established GM lines and matings required for specific experiments. For maintenance purposes we have assumed that four regularly used mouse lines will be maintained throughout the course of the project. We estimate that GM lines engineered to modify molecular factors implicated in X chromosome inactivation will result on average in the need to maintain a further 4 experimental mouse lines. The calculations take into account that during maintenance, only one of the two available copies of a gene is modified (referred to as heterozygous), reducing possible harmful effects. In some instances we make use of genetic modifications that are engineered to manifest only when early embryos are exposed to an inducing signal (a hormone or drug given to the mother). In these instances the GM mouse line can be maintained with both copies of the gene being in the unmodified state.
Calculations for required breeding of experimental mouse lines considers that each line an average series of experiments comprised of crosses within the line (x2), and with different GM mouse lines (x6).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

A thorough literature search is conducted to ensure that experiments that are undertaken do not duplicate reliable data that is already published or present in data/resource repositories. Principles of experimental design are applied in order to minimise the number of experiments required to achieve reasonable statistical significance and data reproducibility.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Where possible we will import previously generated GM mouse lines from colleagues/collaborators/resource centres. To avoid unnecessary breeding/maintenance of strains we will archive (embryo/sperm cryopreservation) an estimated 6 lines in the course of the project. We will supply cryopreserved embryo/sperm for defined lines to other investigators on request in order to reduce duplication of animal experimentation. All vasectomised males are shared between several groups to avoid duplication and to ensure males are not singly housed for large amounts of time.
Stock levels of mouse strains will be set to minimise animal breeding whilst at the same time ensuring that given strains are not lost. Trained animal house staff will carry out the majority of breeding and maintenance, constant contact and instruction will ensure the colonies are maintained at the correct levels.
Tissues/embryos, DNA/RNA and protein samples obtained from experimental animals/embryos are preserved, usually by storing in liquid nitrogen or at -80c, to allow where scientifically justified, use in other experiments by ourselves or other scientists who may request such reagents.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mouse is the system of choice for experimental genetics/genetic modification in mammals and represents the only appropriate animal model for studying the molecular mechanism of X chromosome inactivation. The techniques used here are the most refined at the moment.
Vasectomy:- Animals are expected to suffer no more than moderate harm due to the nature of the scrotal sac method which is the most refined method for this procedure. Where available and cost efficient, we will acquire genetically altered or naturally sterile males that don't require vasectomy. The ability to buy vasectomised males from a commercial supplier is also an option but only if the most refined method is used in their production.
A surgical procedure termed laparotomy is routinely carried out on pseudo-pregnant recipient female mice (after mating with sterile males) for the implantation of embryos during production or rederivation of genetically altered animals. The procedure can be performed on one (unilateral) or both (bilateral) sides of the abdomen depending on the number of embryos requiring implantation, the stage of development at implantation and the number of pseudo-pregnant recipient mice available. An alternative non surgical procedure termed transcervical transfer can be carried out but only on embryos at a specific late stage, referred to as blastocyst.
Superovulation by hormone injection is the standard method to collect the most oocytes and zygotes from the least amount of females. The appropriate concentration of hormone is age, weight and strain dependent, having been optimised in studies over many decades.

Under breeding and maintenance GM mice are not expected to suffer any more than mild pain.
Expected actual severity is sub-threshold. Adults that do show any harmful effects will be culled.
Administration of substances will be carried out by the most refined method available, usually orally.

Why can’t you use animals that are less sentient?

We analyse the consequences of specific genetic modifications for X chromosome inactivation in early mouse embryos. X chromosome inactivation only occurs in mammals so less sentient model organisms are not an option. The embryos need to be produced and maintaining these lines enables us to do this. The majority of the embryos we produce are collected from humanely killed females prior to stages when embryos are considered sentient. Cell lines are derived from early embryo stages and occasionally from humanely killed adult animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will keep up to date with all surgical procedures and their refinements and will attend any relevant workshops.
Laparotomy surgery will cause moderate suffering. Non surgical implantation through the cervix may cause distress if the handler is not as proficient but is not expected to cause harm. Animals are expected to make a rapid and unremarkable recovery from the implantation procedure. Analgesic agents will be administered.
Animals to undergo surgery are given 7 days to acclimatise before procedure starts.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery.
Code of practice for the housing and care of animals bred, supplied or used for scientific purposes

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By regularly attending the NC3Rs days available.
Regular contact with transgenic groups within our institution allow us to update each other with advances in techniques and training.
Departmental animal welfare meetings held termly allow us to communicate any 3Rs implemented within our group and invited members including NACWOs and NVS ensure that any new advances are disseminated.

Home Office

NON-TECHNICAL SUMMARY

65. GENE AND CELL THERAPIES FOR ISCHAEMIC DISEASE

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

pericytes, gene therapy, vascular grafts, angiogenesis

Animal types	Life stages
Mice	juvenile, adult, aged

Retrospective assessment
The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to find new cures for patients suffering from heart attacks and poor circulation in the legs.
A retrospective assessment of these aims will be due by 21 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cardiovascular disease caused by the narrowing of arteries and capillaries that provide oxygen and nutrients to the heart, brain, and legs, is the number one killer in the UK. Current treatments reduce the risk of death after a heart or a brain attack and delay the need of foot amputation as the last resource to save the life of patients with very poor circulation to the legs; however, many patients continue to have a poor quality of life. This is because current treatments are not enough to create new blood vessels around the occluded ones. In addition, grafts used by surgeons to create a new route around the blocked section of the artery tend to occlude after few years from implantation.

What is needed is a definitive solution for building up new blood vessels of different diameter, ideally large arteries to carry blood around the narrowed artery and capillaries to transport oxygen and nutrients to the suffering tissue. To reach this goal we will investigate new methods based on:

3) Testing drugs that can encourage the formation of new arteries and capillaries. For instance, using a protein which protects people who live a long life (centenarians) from suffering from blood vessel occlusion as they become old.

4) Producing artificial tubes containing human cells to create grafts like a real artery

What outputs do you think you will see at the end of this project?

In the medium term, 5 years from now, we expect that this research will demonstrate the proposed solutions are safe and capable of being done. We also expect to obtain new knowledge of how these treatments work. We will publish the results in medical journals where the acceptance for publication is warranted only after careful evaluation by expert reviewers. This will be a demonstration that the research is novel and accurate and has an important medical impact.
In the long term, the project aims to make these new cures available to the patients. This will require (1) preparation of documentation of main findings supporting efficacy and safety, (2) approval from agencies responsible for introduction of new medical treatments.

Who or what will benefit from these outputs, and how?

In the short/medium term, during the next 5 years, the work will generate new information about diseases that cause heart attack and poor circulation to vital organs in the body. We will pay particular attention to vascular cells that have not been investigated sufficiently in the past, such as pericytes, which are regenerative cells lining around the small and large vessels. One major goal is to incite the pericytes to act as building blocks to rebuild new and well functioning blood vessels. We will also work in the lab at producing new tube grafts

containing pericytes and other vascular cells. We expect that, once implanted, these grafts containing human vascular cells will be superior to the ones used by surgeons to bypass blocked circulation.
In the long term, the work is expected to lead to clinical trials in patients. In due course, the work will be of direct benefit to patients and reduce the burden on the national health system and society caused by the disabling effects of vascular disease.

How will you look to maximise the outputs of this work?

The full demonstration that the proposed methods are valid and applicable to patients require skills and expertise that cannot be found in a single laboratory. We will therefore collaborate with other investigators, including doctors, surgeons, and scientists, to achieve the best results.
Clinicians are the best partners for maximising the impact of our research toward patients' benefit, but also to inspire new ideas from the bedside back to the bench. Scientists, with whom we collaborate, can bring new technologies and methods to the research.
We have established contacts with several small and large pharmaceutical companies (where some of our previous research fellows have relocated) and consult them frequently to see if they can be interested in helping us to develop our research and make faster the application to the clinic.
Results will be disseminated through scientific publications and presentations at national and international meetings. This is the most effective method to speed up the progress of science among the experts in the field. We will make sure that methods and results in publications contain enough details for other scientists to repeat the experiments and confirm or extend the acquired knowledge. The data will be communicated, either they confirm (positive results) or reject (negative results) the initial hypothesis. The report of data will follow the current international guidelines.
We have extensive experience in communicating the results through press release, having had articles covered in national news media (radio, television, and newspapers). The team is also experienced in communicating with lay persons through seminars to patients' forums and public lectures. We will use all the above methods to communicate results to the public and patients' associations after consultation with the Research and Development office.

Species and numbers of animals expected to be used

- Mice: 1350

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use mice, which are the mammals with the lowest neurophysiological sensitivity suitable for these studies. Wherever possible, we use the least severe model for our investigations. The disease we want to investigate occurs during adulthood and advanced age, which requires using adult and older models.

Typically, what will be done to an animal used in your project?

The animals will be subjected to surgical procedures to create the condition of blood flow blockade to the heart or limbs as occurs in patients. In other experiments, animals will not be operated but the natural evolution of vascular disease will be evaluated. Treatments will be given through local injections or systemic route. The

duration of experiments will last from 2 weeks to 4-6 months, and we plan to use ~1350 animals during the 5 years of the project.

What are the expected impacts and/or adverse effects for the animals during your project?

Transitory pain after surgical induction of ischaemia will occur like that experienced by patients with a heart attack. Occasionally, we expect loss of weight and difficulty in breathing caused by heart failure and poor ambulation due to reduced blood flow to the lower extremities occurring for the maximum duration of 2 weeks.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

50% mild, 25% moderate, 25% severe.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 21 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We will be using mice for these studies because the complexity of cardiovascular disease and the efficacy of proposed treatments cannot be effectively tested in non-animal systems. However, preliminary studies of cell functions will be carried out before engaging with animal studies. In addition, simulation experiments are planned.

Which non-animal alternatives did you consider for use in this project?

Cellular models. Simulation of blood flow alterations using theoretical modelling.

Why were they not suitable?

Because the final demonstration of efficacy requires the complexity of a living organism.

A retrospective assessment of replacement will be due by 21 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken

to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Based on an average number of 30 animals per experiment for a total of ~45 experiments in 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Well designed and correctly analysed experiments can lead to a reduction in animal use whilst increasing the scientific validity of the results. To this aim, we constantly refer to the NC3R's Experimental Design Assistant as a guideline.
Here, we summarise the main steps considered in the experimental design to reduce the number of animals, control bias and ensure that the results are scientifically valid.

10) Pilot studies comprising a small number of animals to generate preliminary data and/or allow the procedures and techniques to be solidified and “perfected” before large-scale experimentation.

11) The minimum number of needed subjects will be calculated based on the expected average benefit and the expected variability of the benefit (assessed from the literature or pilot studies).

12) The allocation of animals to different groups of treatment will be at random: 1) to avoid biases, 2) to guarantee that groups have the same probability to receive a treatment, and 3) to control experimental variability. When planning attribution of animals to groups, the assignment of animals at random to different groups and sub-groups will be improved by dividing animals in blocks to achieve minimal variation.

13) We prefer to use genetically selected strains of mice because they show a more homogeneous response to the disease, meaning lower variability will allow a reduction in number of animals needed.

14) Important variables as sex, age and weight of the animal should be similar among the groups, again allowing reduction in variability.

15) Our facilities provide state of the art in environmental enrichment. We ensure that all the animals are exposed to the same enriched environment.

16) Collection of data will be done in a manner that the treated and control group have their measures collected at similar time and by the same investigator.

17) Biases will be avoided by ensuring that researchers analysing experimental outcomes are unaware of the treatment received (blinded) until the final statistical analysis.

18) Data will be treated according to the principle intention-to-treat, where all participants who are randomised are included in the statistical analysis and analysed according to the group they were originally assigned, regardless of what treatment (if any) they received

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We based our optimal number of animals and experiments based on experience in previous successful projects, pilot data and computer modelling. We also referred to the NC3R's Experimental Design Assistant to ensure that only the minimize number of animals are used.
We will use the best available technology and make sure that all the equipment is regularly calibrated for precise measurements.

A retrospective assessment of reduction will be due by 21 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are the less neurological developed species that can be used to test the therapeutic interventions we hope to progress into the clinical application. We will use the best measures and practices to attenuate pain, discomfort, infections, and stress. We will adopt refined microsurgical techniques to minimise the adverse effects of surgery. All animals will be carefully monitored after surgery and recorded individually. Wherever possible animals will be group housed and provided with enriched environment. Interventions to assess pain and suffering are refined to provide the maximum benefit for restoring wellbeing without interfering with the physiology of the animals. Therefore, observation frequency is calibrated to the risk of adverse events, which is highest during post-operative surgery. The definition of stress and pain severity is addressed using sensitive scales that have been elaborated for specific use in the specific protocol, for instance to identify low level of pain in mice with heart attack or occluded leg arteries. We also designed the protocol in a way the goal of the research is reached before animals reach the most severe outcome.

Why can’t you use animals that are less sentient?

Less sentient animals, e.g. fish or reptiles, cannot be used because do not reproduce the type of response a human being put in place following injury of the adult cardiovascular system.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have monitoring charts and post-operative charts in place which will be adjourned and improved as new evidence emerges from real experimentation or the available literature. Where appropriate, the animals will be trained to drug administration regimen associated with the lowest possible stress.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Surgical procedures will be undertaken in line with the recommendation set out in the LASA and institutional guidelines for aseptic surgery. Injection and drug administration will be conducted in line with NC3Rs

recommendations.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep informed about advanced in 3R by reading the literature, attending courses and webinars. In addition, we will attend at least 1 meeting every year on animal welfare, such as the RSPCA/UFAW Rodent and Welfare Meeting or other 3Rs symposium.
A retrospective assessment of refinement will be due by 21 April 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

66. Generation of antibodies to detect hazardous agents and their simulants

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (g) Forensic enquiries

Key words

No answer provided

Animal types	Life stages
Guinea pigs	adult
Mice	adult
Rabbits	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to generate antibodies to enable the detection of hazardous agents and their simulants.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There are a number of dangers posed by “hazardous agents” including, but not limited to, adverse effects on human/animal health, contamination of the environment and damage to infrastructure. These hazards can include disease causing bacteria and viruses, e.g. those that can cause deadly illnesses such as meningitis or pneumonia. They may also include chemicals/toxins, the properties of which may, as with biological hazards, pose a risk to health of one or more of the following: humans, animals, aquatic-life, crops and naturally-present fauna and flora.
Knowing whether a person, animal or plant is infected with, or exposed to, these hazardous products, or whether the environment itself is contaminated, is very important in mounting an appropriate response, whether that be a targeted therapeutic treatment or wider-scale environmental decontamination. Knowledge of the presence of dangerous substances and disease-causing micro-organisms is also very important in public health monitoring.
Antibodies are able to be harnessed to identify the presence of a specific substance they have been “raised” to detect. The antibodies produced in this work will be produced to identify specific dangerous organisms/substances and help facilitate the research into and production of: therapeutic treatments, detection and decontamination methods and health surveillance, among other benefits.
Antibodies can also be used in a laboratory-setting in experiments designed to better understand hazardous organisms/substances. These antibodies can facilitate research at a fundamental level, producing publications and high quality research.
New, better antibodies will have great benefit, both furthering fundamental research and improving technologies that involve using antibodies to diagnose, detect and study health hazards.

What outputs do you think you will see at the end of this project?

A key output will be antibodies which can be modified to give off a measurable signal to detect and identify hazards discussed in the “why is it important to undertake this work?” section.
Antibodies produced under this licence will target (and therefore identify) certain specific hazards. An example would be developing antibodies to identify a disease-causing micro-organism which poses a public health risk. The output would be antibodies which can be used to facilitate research into this organism, through use in, for example, laboratory-based testing or screening. Antibodies can be incorporated into diagnostic or environmental detection devices pertaining to these hazards.
Another output will be cells (called “hybridomas” made from collection of animal tissue on a single occasion) which can be stored in cryo-preserved banks and cultured in flasks to make target-specific antibodies as a replacement to obtaining antibodies directly from animal serum.

Development of a methods to produce antibodies with less (and potentially in some cases without), animals will be pursued under this licence.

Who or what will benefit from these outputs, and how?

Correct use of antibodies, in the right scenario, can provide many benefits. Antibodies targeted to bacteria, for example, can be used in a research setting to study specific organisms and publications can be derived from this work, aiding in the diagnosis and treatment of infection. This may benefit industrial and academic research groups.
Applied uses of antibodies include drug discovery, antibody therapy, detection of substances in the human bloodstream or environment, screening for contamination (e.g. in commercially produced food products or scientific samples) and in a diagnostic setting whereby public health threats can be identified/monitored. Academic research groups, and biotechnology and pharmaceutical companies focussed on research into combatting risks from disease-causing and/or dangerous microorganisms/chemicals are examples of organisations which will benefit from antibodies.
In all the above cases, successful utilisation of antibodies to combat/prevent adverse effects of hazardous substances can have a downstream effect that will benefit any individual with actual or potential exposure to, or infection with, these hazardous substances.
Benefits of the antibodies produced in this licence are likely be realised in the short to medium term as they are used in experiments and assays (experimental tests or tests using a device). Cell lines, which produce antibodies, will be retained by the group to help our own work. Cell lines will also be supplied to other research groups, companies and/or organisations to perform research and/or use in assays.
Antibodies and hybridoma cell lines generated have been historically shared with other researchers, for example, those in academia and industry. This sharing will continue under this licence where collaborations are forged. These antibodies and cell lines can be used to support post/undergraduate projects, help develop therapeutics and facilitate microscopy experiments, for example.
Benefits will be realised within the timeframe of this licence; however cell lines can be stored for decades, therefore the potential for further benefits is enduring into the long term.

How will you look to maximise the outputs of this work?

Some work generated under this licence will be deemed confidential, for a number of reasons. This could include non-disclosure agreements between companies or agreements not to share data given by academic groups. Reports will be made available to any researchers with sufficient, relevant permission and authority to view to documents/data. Where the ability to share data and reagents with other companies and/or organisations is not prohibited because of this, it will be pursued. Where relevant antibodies will be made available for other groups to perform research, which may result in scientific publications. These groups and individuals may include companies, academics and students.
Ultimately, however this licence is to supply antibodies, rather than data and findings, and as such it is not expected that scientific papers will arise directly from the work (though use of antibodies by other groups could support this). Rather, the antibodies and cell lines themselves will provide the multitude of benefits as discussed under the "Who or what will benefit from these outputs, and how?" section. **Species and numbers of animals expected to be used**

- Mice: 70
- Guinea pigs: 6
- Rabbits: 3

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the least sentient animals that can be used to suitably make "hybridomas", where cells from a mouse that has been immunised are collected after humanely killing the mouse, altered to make them able to grow in cell culture and then cultured in a flask. This means that cells from one mouse can enable later production of large amounts of antibody in the flask.
Production of antibodies by purifying them directly from animal serum will only be used when there is no other feasible option. In this instance larger animals, specifically rabbits or Guinea pigs, will be used so that a larger volume of serum can be collected from a smaller number of animals. This will avoid using large numbers of mice. Rabbits, being larger than Guinea pigs, will be more suitable for this purpose. However there are instances where Guinea pigs may elicit a better immune response than rabbits.
Polyclonal antibody production takes a shorter time than monoclonal antibody production, and it is expected that animals for polyclonal antibody production will begin the process as an adult, and be humanely killed before they can be considered "aged" animals. For monoclonal antibody production, an immunisation schedule and long rest period can mean that some animals may be considered aged towards the end of the process. However it is important to note that animals will <u>never</u>: **a)** be bought as already aged animals
b) be purposefully aged
All animals used to generate antibodies must have a mature immune system, which excludes animals younger than those stated in this licence.

Typically, what will be done to an animal used in your project?

For animals being used for either polyclonal or monoclonal antibody production, they will be administered with a primary immunisation (an injection via a suitable route for the animal), followed by blood sampling to analyse the titre (usually around 1 week later). If the titre is insufficient they will receive up to 6 boosts to increase the titre. At this point if the animal is used for polyclonal production it will be humanely killed and antibodies will be purified from the serum. This entire process will generally last 3-4 months.
Should the animal be used for monoclonal antibody production it will likely enter a rest period (required to produce immune cells needed for subsequent work) for >8 weeks, at which point it will receive a prefusion boost (usually an intravenous injection), then will be humanely killed a suitable amount of time later, with its immune tissue used in the production of hybridomas, which secrete monoclonal antibodies. This entire process will generally last around 6-9 months.
Blood samples will withdraw a blood volume no greater than 10% total blood volume on one occasions, and no more than 15% over 28 days. Animals will have a maximum of 8 samples taken and sampling will not occur more frequently than one per day, though it is likely they will be separated by around 1-2 weeks.
Animals <u>are not</u> expected to display symptoms of infectious disease or intoxication associated with any disease-causing or toxic agents used in this licence, as the agent will be suitably **<u>inactivated</u>** before it is administered. This is analogous to vaccination, where an immune response is produced without causing disease. In some instances, animals may be immunised with isolated parts of a harmful organism, in a format which is considered non-hazardous.
In instances where chemicals are used, these will be produced by synthetic chemists with expertise in the substance produced. This will ensure that the substance is suitably inactive so as to minimise the chance of adverse reactions in animals.
In as many instances as possible, immune tissue from immunised animals not used in polyclonal or hybridoma generation will be used to support the development of recombinant methods of antibody production, which can reduce and in some instances replace animal use

What are the expected impacts and/or adverse effects for the animals during your project?

Animals will likely shows signs of distress and discomfort during the collection of blood samples however this should resolve immediately after the sample is taken as the distress is mainly due to the animal being held for blood sample collection.
For animals that are administered with the antigen, there may be local reactions which are generally not painful for example, formation of a nodule at the site. If a nodule bursts, it should close within 24 hours.
Injection of antigens may cause abdominal pain which is expected to be generally mild, extending to moderate on occasion.
Exposure to the antigen should induce an immune response, this can cause animals to feel generally unwell for up to 3 days.
As the number of boosts increases, using the antigen, the likelihood of adverse effects increases, however the number of boosts is capped at 6, with some protocols requiring another single boost after at least 8 weeks.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice: Most (60-80%) of animals are expected to experience a local reaction to injection sites which is expected to be mild. This will typically result in formation of a nodule. In up to 25% of these animals the nodule will rupture, which is considered moderate severity. Due to the smaller size of mice, nodules forming in tissues (after intramuscular injections, for example in the thigh), may have more of an adverse effect on the mouse than in larger animals. All animals will experience at least mild abdominal pain with certain injection sites, in some instances (dependent on the antigen used) this may increase to moderate severity. Some animals may appear generally unwell due to a systematic immune response, this is expected to be uncommon and is often (but not exclusively) associated with use of live (non-hazardous) agents which will be avoided where possible.
Guinea pigs: Most (60-80%) of animals are expected to experience a local reaction to injection sites which is expected to be mild. This will typically result in formation of a nodule. In up to 25% of these animals the nodule will rupture, which is considered moderate severity. All animals will experience at least mild abdominal pain with certain injection sites, in some instances (dependent on the antigen used) this may increase to moderate severity. Some animals may appear generally unwell due to a systematic immune response, this is expected to be uncommon and is often (but not exclusively) associated with use of live (non-pathogenic) agents which will be avoided where possible. The use of live (non-hazardous) agents in Guinea pigs is even less likely than its use in mice.
Rabbits: Most (60-80%) of animals are expected to experience a local reaction to injection sites which is expected to be mild. This will typically result in formation of a nodule. In up to 25% of these animals the nodule will rupture, which is considered moderate severity. Injection of rabbits will not use routes which are likely to cause abdominal pain (as sometimes seen in mice and Guinea pigs). Some animals may appear generally unwell due to a systematic immune response, this is expected to be uncommon and is often (but not exclusively) associated with use of live (non-pathogenic) agents which will be avoided where possible. The use of live (non-hazardous) agents in rabbits is even less likely than its use in mice.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Antibodies are the product of an extremely complex immune response, occurring within organisms that are challenged with an antigen. This process is too intricate to be replicated fully and exactly in a test tube with current technology. Some methods are available which mimic, or adapt certain aspects of this, which utilise fewer animals, or potentially no animals, however none have yet been determined as suitable to fully replace animal-derived antibodies for our purposes.

Which non-animal alternatives did you consider for use in this project?

Display platforms- e.g. phage display, using phage viruses to display millions of combinations of antibodies by having them express a library of different genes. Antibody-displaying phage are screened for interaction with the target. Other display platforms include ribosome and yeast.
Naive libraries of antibody fragments (scFv and Fab)- Pre-existing libraries or randomised antibody fragments screened to look for binding to agents, often displayed on phage
Synthetic aptamers and affimers: Biological molecules selected based on ability to bind an antigen, where randomised DNA or protein sequences (libraries) are displayed and analysed for binding to antigen.

Why were they not suitable?

Display platforms such as phage display: The technology is not advanced enough to replace animal derived antibody production for our purposes. There is promise with this technology, and it is in the process of being compared to traditional hybidoma production (supported by this licence). Limitations of phage display currently being addressed include, insufficient library size to give suitable diversity and optimising screening methods to ensure we get sufficient representation of these libraries. Native pairing of antibody chains can occasionally be an issue. Finally, unless performed de novo, with a pre-existing positive control antibody, some animal derived antibodies may be required for phage display as a screen or positive control.
Naive libraries of antibody fragments (scFv and Fab): Attempts at generating antibodies using naive libraries of antibody fragments (scFv and Fab) have not yielded the selectivity, specificity and affinity characteristics required for use in our detection assays. This is likely due to the fact that these libraries are not large enough, and without a robust enough display method for these libraries, this technology is not suitable for our uses. We continue to watch this technology in the hope that it will become viable.
Synthetic aptamers and affimers (biological molecules synthesised to bind to certain agents): Previous tests has demonstrated these are unsuitable at their current technology level. Affimers have been demonstrated to not bind to antigens for long enough, while both technologies can have bias in how the libraries are derived. Being smaller molecules also makes them very difficult to apply in certain assay formats with current technology.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals to immunise to each target is decided through: previous similar data generated in house, other empirical data, subject matter expert input, NACWO/NVS input. Decades of immunisation reports exist which we can draw on to make decisions, along with knowledge of numbers of animals required for previous, similar targets. A statistician was consulted on the animal numbers used in this licence and how these are derived.

The numbers stated directly in the Objectives section are based on the minimum number of animals expected to generate a sufficient amount of immune tissue for hybridoma generation or, where relevant, recombinant methods. Where recombinant methods, are used, they may be more efficient than creating hybridomas, thus the numbers of mice used are decreased to reflect this.
The number of animals required for polyclonal production is based on the amount of antibody needed compared to the volume of serum produced by a rabbit/Guinea pig and the expected concentration of antibody in the serum. The minimum number of animals is selected to obtain the amount of serum, and therefore antibody, needed.
In all the above instances, if a target is expected to produce an excellent immune response, based on previous experience and/or open literature, fewer animals may be used in antibody production as the chance of obtaining a good panel of monoclonal antibodies is increased. It is important to recognise however that downstream hybridoma efficiency can also have a large impact on the success of the work.
The remainder of the animals are to cover the maximum number required for forecasted future work, based on the workload that is expected, split across monoclonal and polyclonal production. When forecasting the number of animals to be used, the "worst case" is used where the maximum number of requests for antibody are pursued.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Previous similar antibody production work and statisticians were consulted to identify a minimum number of animals, which was used to obtain a sufficient panel of hybridomas for monoclonal antibody production or amount of serum for polyclonal production.

Improvements to the downstream processing of mouse tissue are currently under investigation; methods such as gentle mechanical and enzymatic separation of spleen tissue to obtain splenocytes more efficiently and B-cell enrichment, which may lead to less mouse use in the future.
Where possible multiple animal groups all being immunised to the same target are started with an approx. 1 month stagger. If the immunisation is much more successful than expected in earlier groups, and it is decided that not all animals that were estimated to generate sufficient immune tissue are required, then ordering of the full anticipated number of animals will be re-evaluated. This will mean less animals are used.
Deep tissue sequencing technology, as well as antibody display library technology are being investigated to reduce animal usage in antibody production. By sequencing the immune tissue, the genetic code of antibodies can be identified and antibodies produced recombinantly (i.e. synthetically). Less animals will be required using these methods than traditional hybridoma production.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For monoclonal antibody production, spleen cells are primarily used, however where possible, immune cells will also be harvested from the mouse femur, for example.
Best endeavours will be made for remaining tissues from rabbits or Guinea pigs immunised with antigen to be used to support the development of new antibody production techniques which require fewer animals, or potentially none, in some circumstances, making possible the identification of rabbit or Guinea pig monoclonal antibodies (recombinant) without the risk of needing to perform repeated rounds of mouse hybridoma generation in order to discover reagents suitable for filling our requirement

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs

(harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice: An animal model with one of the lowest capacities to feel pain, suffering, distress, or lasting harm but still generate suitable immune tissue. Species lower than a mouse generally do not have complex /suitable immune systems to generate required antibodies.
Rabbits and Guinea pigs: For experiments where larger volumes of serum are required (i.e. polyclonal antibody production), the use of fewer larger animals will be preferred over the use of a higher number of smaller mice. The species selected are some of those with one of the lowest capacities to feel pain, suffering, distress, or lasting harm but still generate a suitable immune response with a sufficient amount of circulating serum. Usually rabbits would be preferred as a species with a larger serum

volume compared to alternative small animals, unless there is evidence to demonstrate a good immune response associated with a different species (i.e. in some circumstances Guinea pig). The increased serum volume in rats over mice does not have the magnitude to make it as useful an alternative as Rabbits and Guinea pigs, additionally rat antibodies are more likely to cross-react with mouse antibodies.
Traditionally, polyclonal antibodies would be generated in larger animals, such as donkeys or sheep. These are not used here as: **a)** that volume of serum is not required for the small scale amount of polyclonals we will use and **b)** smaller animals can be more easily kept in contained conditions.
Guinea pigs are utilised in protocol 1 as an excellent immune response has been demonstrated towards the relevant immunogen which will be used in this study. It is decided that due to the increased likelihood of getting a good immune response, Guinea pigs will be used.

Why can’t you use animals that are less sentient?

Animals less sentient than mice (e.g. insects, which lack the immune memory of mammals) are not suitable for monoclonal antibody production. A mouse is the least sentient animal model with a good chance of generating hybridomas.
A functional, mature immune system is required, with immune schedules taking place over months. This prevents use of animals in immature life stages, when they would have reduced sentience (e.g. prior to two thirds of the way through gestation) and under terminal anaesthesia.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

A less distressing form of administration of antigen will always be chosen first (e.g. subcutaneous in mice), unless it does not, or is reasonably expected not to, elicit a suitable immune response.
Where suitable, subcutaneous administration will utilise a "scruffguard" or similar device, whereby the mouse is placed on a spongy surface and the "scruffguard" is placed over the animal to allow easier scruffing and injection of the antigen. This avoids having to restrain the animal and scruff at the same time, which is more distressing on the animal.
For all animals, there will be vigilance for potential use of milder adjuvants which may decrease the adverse effects experienced by animals being immunised. There will be limits on the number of times an animal is immunised, and injection sites will be checked daily if any adverse effects are seen.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Available guidelines on the NC3Rs website regarding blood sampling will be followed. This references "A Good Practice Guide to the administration of substances and removal of blood, including routes and volumes" by Diehl et. al. This will also be followed regarding the administration of antigen.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Updates on advances in the 3Rs are regularly distributed by our Named Information Officer. The licence holder

is actively engaged in our establishments Animal Welfare and Ethical Review Body (AWERB). Any appropriate advances will be discussed with our veterinary staff and, where appropriate and compatible with the scientific aims of the project, these advances will be incorporated.
A watching brief will be kept in working towards adopting methods to replace and reduce animals.

Home Office

NON-TECHNICAL SUMMARY

67. Genes and essential nutrient influences on behaviour.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Methylation, Circadian rhythms, Methionine

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how essential nutrients and their metabolism can influence our physiology and behaviour, and in particular our biological rhythms.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

We eat food to stay alive; our body needs energy to keep functioning. Beyond simply providing energy, some nutrients dramatically influence how our body functions. This is especially true for vitamin B9 and the essential amino acid methionine, whose deficiencies can cause many pathologies including aging, diabetes, and neurological problems. These deficiencies can either arise from a poor diet or from genetic mutations that are often undiscovered until severe symptoms occur. Yet, the mechanisms underlying how these diseases occur are poorly understood.
In our body, vitamin B9 and methionine are involved in the metabolism of methyl groups. Methyl is a small chemical group, composed of only one carbon atom linked to three hydrogen atoms, which can be attached to other molecules in the cell, including DNA, RNA or proteins. The addition of a methyl group, or *methylation*, is a mechanism by which our body can regulate the function of virtually every genes, depending on what the methyl is attached to. Low amount of methionine or vitamin B9 in our diet, or mutations in genes involved in their metabolism, will inhibit methylation reactions and cause wide-ranging disruptions.
Our research has previously revealed a link between methyl metabolism and the body clock in many organisms from bacteria to humans. However, this research was based on *in vitro* experiments, using cell cultures. The nature of this link and how it is regulated is unknown. Using mice, whose methyl metabolism is virtually identical to ours, we seek to define which methylations are linked to our body clock, and whether and how they are regulated by our diet.
In addition to further our fundamental understanding on how nutrients can affect our body, this research will also provide potential new targets for the treatment of diseases related to methionine deficiencies.

What outputs do you think you will see at the end of this project?

Discoveries made during this research will contribute to our understanding on how our bodies work at the fundamental level but will also provide insights into two clinically relevant themes: nutrients metabolism and the body clock.
The body clock is clinically relevant because our life-style and 24-h society have detrimental consequences on our biological rhythms, leading to poor general health and increased incidence of cancer, cardiovascular and metabolic diseases. Interestingly, deficiencies in essential nutrients when unnoticed cause similar pathologies. These deficiencies can be caused by a poor diet but can also originate from genetic mutations.
The evidence accumulated so far suggest that these two themes, clock and diet, are intimately linked in our body; understanding such a link will therefore increase our understanding on how our body functions and responds to stress, and provide potential new avenues for the treatment of related pathologies.

Who or what will benefit from these outputs, and how?

- **Academic beneficiaries**

At the local and (inter)national levels, results from this project will find beneficiaries in fundamental as well as in more applied academic fields.

- **Society**

This research seeks to understand the link between our diet and our body clock, how they regulate each other, and what pathologies occur when this link is disturbed.

How will you look to maximise the outputs of this work?

Positive and negative research results will be published in Open Access journals, and data produced by this research will be deposited in repositories (e.g. NCBI's Gene Expression Omnibus, EMBL's Proteomics Identifications database) when appropriate. Research results will be presented at (inter)national conferences and host laboratories. Scientific papers will be accompanied by media releases to reach the general public. Collaborations with (inter)national laboratories have already been set up and will develop further in the course of this research. When appropriate, tissues and data will be shared with direct collaborators.

Species and numbers of animals expected to be used

- Mice: 11500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In the first part of this research, the mice will be genetically modified but will develop and grow healthily just like normal mice. A drug called tamoxifen (used in the chemotherapy of breast cancer) can then be added to the food of these mice, usually at the adult stage, which will cause the inactivation of a selected gene. This allows the physiological role of that gene to be investigated, without risking potential negative consequences on the development of the animal.
In the second part of this research, animals used will be wild-type and healthy adult mice that will be given food lacking essential nutrients such as methionine and choline, whose insufficient intake has been associated with hepatitis in humans. However, how these nutrients are used in our body, and what processes they regulate, is not well understood.

Typically, what will be done to an animal used in your project?

A typical experiment in our project will involve monitoring mice in a cage containing various items to interact and play with, notably a running-wheel that is connected to a computer to measure the activity of the mouse, or a system that measures when and how much the animal eats and drinks. Since these behaviours are controlled by the mouse body clock, when the mouse is in complete darkness these rhythms in wheel running and drinking can be quantified and used to measure the internal biological rhythms of the mouse. The effects of gene inactivation and nutrients deficiency on these behaviours will be studied. In addition, some animals may be killed by a Schedule 1 method in order to identify the molecular mechanisms triggered by these nutrients, or lack thereof.
The precise procedures that will be performed on the animals in this project, will be the following.

Part 1:

1) Animals will be genetically modified.

2) Animals from 1) will be provided with a diet containing the drug tamoxifen, or may be given tamoxifen via gavage (direct administration into the stomach through a tube) or subcutaneous injections (only if the diet method does not yield the expected results).

3) Some animals from 1) and 2) will be single-housed and monitored for biological rhythms under normal light/dark cycles and constant darkness for up to 2 months.

4) Some animal from 1) and 2) other than the one used in 3) will be single-housed and undergo physiological monitoring and/or imaging using non-invasive specialist equipment for up to 6 weeks.

5) Animals may also have small blood samples collected (microsampling), and their ear may be notched for identification.

Part 2:

6) Animals will have the composition (decreased levels of essential nutrients) of their food changed for a duration of up to 3 months.

7) Animals from 6) will be single-housed and monitored for biological rhythms under normal light/dark cycles and constant darkness for up to 2 months.

8) Animal from 6) other than the one used in 7) will be single-housed and undergo physiological monitoring and/or imaging using non-invasive specialist equipment for up to 6 weeks.

9) Animals other than those used in 6) will be fasted for a maximum of 38 hours.

10) Animals may also have small blood samples collected (microsampling), and their ear may be notched for identification.

What are the expected impacts and/or adverse effects for the animals during your project?

Inactivation of candidate genes may cause chronic pathological consequences such as weight loss, anaemia and an inefficient immune system.
Single-housed mice can experience some level of stress and anxiety, that may be more pronounced when it is in constant darkness for 2 months.
The diets lacking methionine and choline, when given for a period of up to 3 months, are likely to cause health issues in mice as they do in humans, notably weight loss and hepatitis, although this will not be allowed in this project since healthy mice are needed.
Veterinary help will ensure mice showing these symptoms are treated appropriately.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Sub-threshold: 50%
Mild: 40%
Moderate: 10%
Severe: 0%

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

These studies are aimed at understanding how the metabolism of essential nutrients can influence our physiology and behaviour. Therefore, an animal showing a complex array of human-like behaviours is needed.

Which non-animal alternatives did you consider for use in this project?

Human and mouse cell cultures will precede all investigations involving animals.
We have also considered simpler organisms, including fish, flies and even plants and bacteria.

Why were they not suitable?

While cell cultures are key to study molecular mechanisms, investigating physiology and behaviour can only be done with a complete organism.
Less complex organisms do not exhibit the same array of human-like behaviour that mice do, and do not have the same dietary requirements.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

11,500 is the maximum number of animals estimated, but the actual number of animals used is likely to be lower. This number was estimated on the basis of the number of different genes that will be investigated, each of which requires a separate genetically-modified mouse line and respective controls. An independent statistician has been consulted on the number of animals required to achieve the objectives of our research.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The experimental designs were approved by an independent consulting service.

We are working with the NC3Rs experimental design assistant to help us ensure the experimental designs are appropriate.
Animals studied will have the same genetic make-up and age to reduce variability.
To avoid experimental bias, random allocation of mice to treatment groups or to cage number and position within the animal house will be carried out, and the investigators assessing the outcomes of experiments will be blind to the nature of the groups to be compared.
Animals of both sexes will be used, which ultimately will decrease the total number of animals used.
Typically, randomisation will be carried out using a computer's randomize function to avoid human bias.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

When applicable, *in vitro* experiments using cell cultures will be carried out to first test the validity of our hypotheses before deciding whether *in vivo* experiments should go forward.
Pilot studies will be used; they will enable us to determine the most efficient and least stressful methods, as well as to obtain a first idea of the effects triggered by the procedures.
Another benefit of running pilot studies is that it will allow a more accurate estimation of the required number of animals.
Genetically modified animals expressing the luciferase gene as a reporter for molecular circadian rhythms *in vivo* will be measured longitudinally, at multiple time points, without the need for humane killing of different animals for every time point.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Tamoxifen-inducible gene inactivation allows the physiological role of a given gene to be investigated without risking any negative consequences on the development of the animal. Tamoxifen administration via food intake is the preferred method because it is not invasive and based on the animal's own volition.

Why can’t you use animals that are less sentient?

Immature life stages are not appropriate because inactivation of the genes studied here cause early developmental arrest, and less sentient animals do not show complex human-like metabolism and behaviour.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinements throughout the project

In relation to how the welfare of the animals is affected by the procedures, monitoring may increase or decrease in frequency and details. Should the animals develop pathologies to a level that is more serious or earlier than anticipated, increased care, under the veterinary surgeon's supervision, will be provided and protocols will be updated accordingly.
As much as possible animals will be acclimatised when transferred to a new environment.
When animals are single-housed, environmental enrichment will be provided to alleviate potential anxiety due to social isolation.

Animals of both sexes will be used, which will increase the relevance of our research results.
Animals kept in constant darkness will be killed by a Schedule 1 method in the dark, using night-vision goggles, to prevent acute stress to the animal and avoid the effects of light exposure on the animals biological rhythms.

The licenced personnel will be trained to use the most refined methods of mouse handling and husbandry, providing environmental enrichment in the cages so that the animals can display an appropriate range of behaviour as in the wild.

Refinement specific to the first part of the project

Pilot studies have been set up to be able to determine the most refined methods in the administration of substance such as tamoxifen. A common problem of tamoxifen-containing diet is their low palatability, decreasing food intake, causing weight loss, and lacking efficiency. To refine this, the highly palatable sucrose will be added to the diet to promote food intake.

Refinement specific to the second part of the project

Pilot studies have been set up to be able to determine, with a view to stop or prevent, the effects of methionine/choline deficient diet on the animals' health.
Unfortunately, commercially available methionine/choline-deficient diets have been optimised to quickly induce fatty liver diseases, notably by adding sucrose and polyunsaturated fatty acids. However, we will not include these "improvements" in an attempt to prevent or delay the incidence of fatty liver diseases while still allowing the specific roles of methionine and choline to be detected.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes published by the Home Office (2014) will be followed.
The LASA guidelines for record keeping will be enforced to all personnel working on the project.
A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes (Diehl et al., 2001) will be followed whenever a procedure requires administration of substances and removal of blood.
The ARRIVE guidelines will be followed when reporting research results using animals.
These best practices will evolve whenever these guidelines are updated.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through discussions with colleagues and named persons at the institute, through keeping up to date with recent discoveries in the field, and through frequent visit to the NC3Rs website looking for resources. We have an account with the NC3Rs and receive frequent updates by email. The NC3Rs regional programme manager, available on site can be consulted about recent advances.

Home Office

NON-TECHNICAL SUMMARY

68. Genes and lineages in blood and immune cell generation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Stem cells, Hemato-immune system, Lineages, Gene regulatory networks, Mouse development

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to breed and analyse genetically altered mice to further our understanding of the cell lineages and gene regulatory networks involved in the generation of blood and immune cells during embryonic development.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Blood and immune cells are critical for the health and survival of an organism, with red blood cells transporting oxygen and white blood cells of the innate and adaptive immune system fighting invading microorganisms. The process by which blood stem cells and the layered immune system are generated during embryonic development is poorly understood. To understand this process in sufficient detail to develop and improve therapeutic applications for blood and immune disorders, it is important to understand how these cells are born during embryonic development. Indeed, the notion that all immune cells are derived from blood stem cells has recently been challenged. It has become increasingly clear that several types of immune cells that display specific functions later in life are generated independent from blood stem cells early in embryonic development. The precise cellular origin of these immune cells remains unclear. Here we aim to trace the origin and development of blood stem cells and immune progenitors in the mouse embryo, begin to map their respective contribution to the adult blood and immune system, and characterize the gene regulatory networks underlying their generation. Our studies are expected to contribute important new insights into blood stem cell biology and innate immune cells. **What outputs do you think you will see at the end of this project?**

The output of this project is new knowledge about the cellular and molecular mechanisms that underlie the initial generation, maintenance, and expansion of blood and immune cells in embryonic development. Within the current interest in regenerative medicine, it is important to obtain a thorough understanding of how adult stem cells and the organ systems they maintain are generated. The elucidation of the gene regulatory network and signals underlying blood stem cell emergence will form a valuable benchmark for future studies into how this network is perturbed in leukemia. In addition, the knowledge obtained from this project will be valuable for the future development of new protocols to generate blood and immune cells in vitro, such as from induced pluripotent stem cells (iPS) and embryonic stem (ES) cells. This would be valuable both for research purposes such as drug development and for the improvement of stem cell-based therapies in for example leukaemia. We expect the output route of this project will be primarily through publication in peer-reviewed journals, through presentations at international and national conferences, through collaborations, and through science events for the public.

Who or what will benefit from these outputs, and how?

Short term: The foreseeable short-term impact is the valuable insights obtained from this project for the field-specific scientific community, whereby data acquired here will form the basis for new lines of work.
Long term: The long-term impact may include the establishment of novel blood stem and progenitor culture and expansion methods/conditions as well as identification of critical genes/gene networks that can be therapeutically targeted.

How will you look to maximise the outputs of this work?

We have a history of successful collaborations all over the world. Through such collaborations we are exchanging project-related knowledge before it becomes public, allowing us to tweak experimental designs and revisit aims and questions of our projects and likewise inform the work of colleagues. In addition, we attend

conferences, symposia, meetings and talks to share our unpublished results.

Species and numbers of animals expected to be used

- Mice: 11600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The study of the development of blood and immune cells in embryonic development requires we use embryos as our experimental model, as the complex developmental processes we study cannot be replicated in the laboratory. We use the mouse as our model as this has many similarities with human for the processes we study. Most of our work involves isolating cells from embryos at different stages of development, that reflect discrete steps in the development of blood and immune cells, for experiments performed outside the animal in the laboratory. To obtain embryos of a particular developmental age we set up timed matings. For this we maintain mouse colonies that carry for example fluorescent reporter genes that mark our cells of interest, or that carry extra or fewer genes involved in the generation of blood and immune cells so we can test the role of these genes in developmental processes. At birth mice are less developed than human babies, so to study stages equivalent to late human development we also study newborn mice. As blood and immune cells still develop in early life, we also sometimes study cell obtained from juvenile and adult mice.

Typically, what will be done to an animal used in your project?

Typically this project involves breeding and maintenance of genetically altered mice and setting up timed matings for embryo analyses in the laboratory. Mice are marked for identification purposes. On some occasions need to perform experiments in the mice. This includes 1) testing the capacity of cells to regenerate the blood of irradiated mice in an assay similar to what happens in bone marrow transplants of patients with leukemia. These so called reconstitution assays take up to 6 months to be sure that the test cells contained blood stem cells. 2) In another assay we administer a gene inducing or deleting substance to a pregnant female. These assays typically need two inherited components to work. As the pregnant female only carries one of them (the other is provided by the father), genes are only induced/deleted in the embryos. The embryos are analysed in the laboratory. 3) Finally, we occasionally label the newly synthesized DNA of cells by administration of a DNA-base analogue that is incorporated in the DNA. As this only happens when cells divide, this assay gives us information about cell division so we can study how this is related to the processes underlying the generation of particular blood and immune cells.

What are the expected impacts and/or adverse effects for the animals during your project?

The vast majority of mice used on this licence will be bred and used for tissue harvest following Schedule 1 killing. For mice that undergo the experimental procedures in this licence are closely monitored for adverse effects (e.g. dehydration as a result of the irradiation, abortion due to the administration of gene

inducing/deleting substances), and killed if adverse effects arise, though these are rarely seen. The in vivo assays involve momentarily discomfort from the injection, but no other adverse effects are expected.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The large majority (approximately 90%) of the animals are only used for breeding and maintenance, including timed matings. The remaining approximately 10% undergoes an experimental procedure. Over 95% of the mice used for breeding/timed matings have an expected actual severity level that is sub-treshold; the remaining up to 5% are expected to experience a mild severity level. Of the mice undergoing an experimental protocol, approximately 45% is expected to experience an actual subthreshold severity level, approximately 20% an actual mild and approximately 35% an actual moderate severity level.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Blood and immune cell generation in the embryo is a complex process that so far cannot be reproduced in vitro. During embryonic development, blood stem cells and other progenitors of blood and immune cells are generated in asynchronous waves that take place at different locations in the embryo and surrounding membranes. The cells generated in these waves migrate to the fetal liver and bone marrow where they generate more blood and immune cells that are distributed over various other organs. Blood stem cells can currently not be robustly generated in the laboratory, and the migration of cells in the embryo can only be studied in situ. We use the mouse as a model organism, as this is the lowest mammalian species with clear similarities to the development of human blood and immune cells. It is not feasible to use human material to study the migration of cells over the embryo as this involved inherited labelling methods not possible in human. Also, the earliest stages we studied are seldomly available in human as they are equivalent to pregnancies of less than 1 month.

Which non-animal alternatives did you consider for use in this project?

We use mouse embryonic stem cell (ESC) cultures and/or human induced pluripotent cells (iPSCs), and cell lines for part of our assays. Overall, we make use of a large variety of in vitro/ex vivo assays wherever possible.

Why were they not suitable?

Although mouse ESCs and human iPSCs can be differentiated in vitro into blood progenitor cells, their maturation in the red and white blood cells found in the blood is often incomplete and importantly blood stem cells cannot be robustly generated in these cultures. The type of blood and immune cells obtained from these models is thought to reflect those found in the membranes surrounding the embryo (the so called yolk sac). So we use this model to study basis processes in yolk sac blood cell generation, in particularly when large cell

numbers are needed for the experiment, for which sometimes cell lines are useful too. Still we largely depend on embryo-derived material for our studies into blood and immune cell generation because of the dynamic changes in space and time that are associated with the generation of these cells.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals is estimated based on the type and number of experiments that can be performed by the laboratory group over a period of 5 years, and the number of animals that needs to bred to perform these experiments. Most of the animals are used in a breeding protocol.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experiments are carefully planned to avoid wastage, and the size of the mouse colony is continuously tailored to meet experimental needs, to avoid surplus. We freeze embryos/sperm to archive lines not in use.
We perform pilot experiments to explore new hypotheses and design the experimental series based on their outcome. We make sure to use the right number of mice to see an effect so it is clear when there is none and experiments can be ended.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use the minimum of stud males and females for timed matings, as we screen the females for estrous so they are likely to get pregnant after mating.
When a few mice of a specific strain are needed, we seek to obtain these from local colleagues to avoid duplicating the colony. This reduces mouse numbers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse is the best studied model for blood and immune cell generation in mammals. It has many similarities with human, a plethora of markers is available for the identification and isolation of different blood and immune cells, and many in vitro assays are available to test cell characteristics. In addition, blood stem cell transplantation assays have been pioneered in mice and are well established and there is a wealth of data to build our studies on. In addition, the multiparity of the mouse makes it feasible to obtain sufficient material for study.
We monitor mice in breeding protocols for aggression by either sex. This is rare, but if it happens aggressors are replaced.
Irradiation can cause temporary discomfort though it is rare in the type of mice we use. Where possible we use outbred strains as recipients, which are more robust than inbred strains. Irradiation occurs in a split dose to minimize adverse effects on e.g. the gut. Irradiated mice are closely monitored for adverse effects of irradiation.
With the administration of gene inducing/deleting agents rare adverse effects can occur which are mitigated by close monitoring. We choose the route of administration that causes least distress while meeting the specific requirements of the particular experiments.

Why can’t you use animals that are less sentient?

Most of our work is done with cells obtained from mouse embryos, which is the least sentient life stage in mice. For repopulation experiments to yield clinically relevant data on blood stem cells, experiments need to be performed in adults as this is the main life stage in humans that undergoes bone marrow transplants as therapy for severe blood-related disorders.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Most mice will not experience welfare issues during their lifetime, apart from occasional mild and transient discomfort or pain associated with marking, administration of substances, or blood sampling via a superficial vessel. Rarely, mice may experience moderate adverse effects that is mostly transient due to irradiation. To minimise this, animals will be closely monitored in the critical 2 week window after irradiation and any animal exhibiting more than moderate transient effects will be humanely killed.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow guidance from NC3Rs and local guidelines based on these. We will follow ARRIVE guidelines for the reporting of animal experiments.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Dissemination of the 3Rs is through discussion at lab meetings, information from the NVS and NACWO, attendance of the termly departmental welfare group at our university and 3R days, the nc3rs.org.uk website. We also aim to find new ways to implement the 3Rs in discussions with animal technicians and colleagues.

Home Office

NON-TECHNICAL SUMMARY

69. Genetics of Down Syndrome

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Down syndrome, Congenital heart defects, Craniofacial development, Neuronal development, Cognitive function

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to identify genes that are needed in three copies to cause specific Down syndrome phenotypes and establish the mechanisms by which they cause pathology.

Potential benefits likely to derive from the project, for example how science might be advanced or how

humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Down syndrome is a human condition caused by an extra copy of chromosome 21. It is a complex disorder with many different phenotypes including leukaemia, autoimmunity, congenital heart defects, craniofacial changes, and cognitive deficits. It is not known which of the ~230 coding genes on chromosome 21 is required in three copies to cause these diverse phenotypes, and thus there are no effective treatments for any of these conditions. We aim to identify the causative genes and establish how an extra copy causes pathology. Knowing this will lay the foundations for the design of rational therapies for the phenotypes of this common genetic disorder.

What outputs do you think you will see at the end of this project?

The main outputs of this work will be knowledge about which genes cause DS phenotypes and the mechanisms by which they do so. This will be published in peer-reviewed journals, and the publications will always be open-access and thus available to all to read for free.

Who or what will benefit from these outputs, and how?

The first beneficiaries will be other researchers working on DS. More importantly, in the longer-term the work will benefit those who are working towards identifying rational therapeutic approaches to alleviate DS phenotypes. Ultimately, the beneficiaries will be people with DS who will have access to therapies that will be developed on the basis of this research.

How will you look to maximise the outputs of this work?

The main outputs from the work will be published in peer-reviewed journals, and the publications will always be open-access and thus available to all to read for free. We will also communicate our work through presentations, by giving seminars at other institutions or through seminars or poster presentations at conferences. Unsuccessful approaches will be discussed openly in appropriate venues, for example at internal meetings. We have an extensive track record of collaboration, helping other groups with more limited experience in these areas of research and sharing all our novel mouse strains, often before publication. We will continue to support such collaborative work. **Species and numbers of animals expected to be used**

- Mice: 24000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The project will use embryos, neonates or juveniles to study developmental processes, e.g. heart development to investigate congenital heart defects. Adults will be used to study physiological processes typical of adult mice, e.g. cognition, locomotor function.

Typically, what will be done to an animal used in your project?

The large majority of mice in this project will be bred and then killed and tissues analysed by, imaging, cell culture,

transcriptomics, proteomics flow cytometry, etc. Some mice may be treated with substances to induce gene deletion or to report cell proliferation. In some case adults will be analysed by in vivo imaging, or in tests of cognition or locomotor activity.

What are the expected impacts and/or adverse effects for the animals during your project?

For the vast majority of mice there will be no adverse effects. For some animals, there will be transient pain when being injected, but no lasting harm.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild – 95%
Moderate – 5%

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The DS phenotypes we are studying – congenital heart defects, otitis media, craniofacial changes, learning and memory deficits, immune dysfunction and locomotor impairment – cannot be studied in vitro. These phenotypes can only be meaningfully studied in vivo.

Which non-animal alternatives did you consider for use in this project?

We considered using human IPSCs from DS people and differentiating these into relevant cell types.

Why were they not suitable?

The use of IPSCs is possible, but very limited – essentially only individual cell types can be studied and the complexity of tissue interactions cannot be replicated in vitro.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimate is based on our current experience of carrying out similar studies under our current project licence, and the number of researchers working on this project which will remain around 5 individuals for the next 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The efficiency of animal usage will be maximised by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. This has been greatly facilitated by a custom-built mouse database in which every breeding pair and every mouse born are recorded and through which we can readily monitor the numbers of mice we hold. Many experiments will require homozygous mutant animals. Littermates of these that are heterozygous or wild type will be used as age- and gender-matched controls. This allows optimal use of mouse numbers generated as well as being best scientific practice for the study of genetic alterations.

The experimental design is always based on using the smallest number of animals that are sufficient to answer the question being posed. We expect, from experience, that 6-8 animals per treatment group should be sufficient to obtain statistically robust results. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from the literature).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding strategies are always set up to maximise the number of useful mice from each litter. Wherever possible we will use multiple tissues and/or fluids from every animal, in order to maximise the data obtained from each mouse. Cryopreservation of gametes or zygotes will be used to preserve mouse strains, thereby obviating the need for continuous breeding and thus minimizing numbers of mice used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mice since these are the mammal with the best studied developmental biology and cognition.

Why can’t you use animals that are less sentient?

Many of the mice we will use will be embryos – these are most suitable for the study of developmental processes, e.g. in the heart. Since we are modelling DS phenotypes, including cognitive deficits, less sentient animals would not be suitable as they are too far away from humans.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For all manipulations, we will adhere to the relevant guidelines that aim to minimize suffering. We examine the animals for signs of pain and discomfort (such as grimacing), providing additional analgesia if appropriate, and

monitor body condition, killing the animals if the distress is likely to be more than temporary. Many of the genetic and physiological manipulations, as well as the administration of substances, including gene inducers and repressors are standard and previous refinements from the literature will be used and added to if possible. For novel types of manipulation, or where insufficient information is available, small-scale pilot experiments are conducted in order to determine the best conditions to obtain a sufficiently robust and meaningful response from the minimum dose, exposure time or treatment. These pilot experiments help to minimize any potential suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Publications from the NC3Rs and the Institute for Animal Technology, as well as relevant articles in scientific journals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay up to date via regular communication with animal facility staff at the host establishment, other scientists in our fields, via e-mail and other updates and publications from, and occasional attendance at meetings held by, the NC3Rs, the Institute for Animal Technology, and the International Society for Transgenic Technology, and through regular visits to their websites:

https://www.nc3rs.org.uk/3rs-resources

https://www.transtechsociety.org/ https://www.iat.org.uk/

Home Office

NON-TECHNICAL SUMMARY

70. Germ cell modification of birds

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (e) Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work

Key words

No answer provided

Animal types	Life stages
Gallus Gallus	embryo, neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of the project is to understand and modify chicken genes using the reproductive cells of chicken. The results from these studies will be useful for understanding bird fertility, poultry production traits, disease resistance, and for the safeguarding of breeds of chicken.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The chicken, a non-mammalian animal, is a valuable model for understanding the genes important to form a healthy embryo. As the most numerous agricultural animal with production reaching tens of billions of birds annually, understanding the genes that control growth, immunity, feeding and behaviour, and reproduction will help us to increase productivity and welfare in poultry farming. This project will also increase our basic understanding of development of reproductive cells in birds. The process of germ cell development is central to reproduction and procreation.
In fact, some of the genes we have investigated using genome editing have caused the sex of the chicken to reverse which shows the importance of the same genes in both birds and mammals.

The knowledge acquired in this project will help understand the reproductive problems that exist in commercial poultry production. Broiler chickens (raised for meat production) are required to be kept on a severely feed-restricted diet to maintain their normal cycle of egg laying. It is possible that understanding how normal reproductive cells form in chicken will help us discover how and why broiler chickens lay double yolked eggs if allowed to feed freely which will improve commercial welfare.

This project also has benefits for bird conservation and the management of poultry genetic resources. Breeds of chickens and specialised lines of chicken (transgenic chicken lines and naturally occurring breeds that have mutations that cause a loss of vision or polycystic kidney disease) are kept as living flocks of animals because no methods exists to bring back a pure breed of chickens from frozen material. This project will develop the methods to make breeds and transgenic lines of chickens from frozen reproductive cells using surrogate hosts. This will have many direct benefits i) lines and breeds of chickens can be protected against loss and from epidemics such as avian influenza; ii) breeding flocks do not need to be kept to maintain a chicken breed or line so the numbers of specialised chicken breeds with defined genetic mutations or transgenic lines can be frozen, 'biobanked', which will support the concept of the 3Rs, this will reduce the breeding numbers of chicken kept for research; iii) flocks and lines of chickens could also be efficiently managed for agricultural purposes, as specific breeds could be frozen then brought back at a later date when needed for food production. It is possible that the techniques developed in this project could be applied to all bird species to aid with their conservation.
What outputs do you think you will see at the end of this project?

The project will help identify the genes that are important for production traits such as rapid growth, increased muscle mass, a heathy gut and a proper feeding appetite. We will also identify the genes and genetic differences that are important during disease infection and for disease resistance to the many poultry diseases, such as avian influenza.

We will also identify genes that are important for the fertility of birds and for chicken egg production.
This project will also develop the methods to cryopreserve breeds of chickens and produce chicken using 'surrogate' host birds. This will reduce the breeding numbers of chicken kept for research. We will also be able to have the mother hens lay eggs containing embryos with genetic changes without the hens having to experience health issues from these genetic changes.

Our results will be published in scientific journals and communicated to poultry research laboratories worldwide.

Who or what will benefit from these outputs, and how?

The public will benefit from this work through safer sustainable food production. Commercial chicken are vaccinated to prevent disease. Our increased understanding of disease pathogenesis will help make poultry resistant to these diseases and improve vaccination programmes or eliminate the need for vaccination, increasing the health of poultry and of human communities that depend on these birds for their livelihoods.

This project also has benefits for poultry conservation and the management of poultry genetic resources. In the future this will also be a benefit for poultry through increased welfare by replacing the need to breed flocks of chicken. In the end, agricultural companies will benefit as their production costs will be reduced and their flocks can be protected from pandemics which can destroy both chicken flocks and chicken breeds.

How will you look to maximise the outputs of this work?

We collaborate internationally and communicate our scientific results at seminars, conferences, 3R conferences, and through scientific publications and poultry news journals. We communicate with other laboratories who are also developing poultry cryopreservation techniques and genome editing techniques and we also speak with regulators internationally at the USDA and FAO through our scientific collaborations. We seek to streamline the pathway to breeding genome edited birds and managing flocks of both normal breeds and special research breeds of poultry and post these results on institute webpages and through institute blog sites.

Species and numbers of animals expected to be used

- Domestic fowl: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The chicken is an appropriate animal model to study avian development, embryology and agricultural traits. It may also prove to be useful in the re-establishment of other bird species from cryopreserved material. The chicken is very useful to study embryonic stages as a fertilised egg can be obtained without surgery on the mother. However, to study gene function, agricultural traits, and disease resistance, we need to use adult birds.

Typically, what will be done to an animal used in your project?

The animal will be blood sampled one to five times during its life. Its production traits will be measured and its behaviour observed. It may be assayed for disease resistance in challenge experiments carried out on other licences with appropriate authority. It may be treated with a drug to change gene expression or ablate cells in the animal. The animal will be humanely euthanised after which phenotypic and genotypic data will be obtained.

What are the expected impacts and/or adverse effects for the animals during your project?

There is a reduced hatching rate experienced for some of the lines of chicken and from manipulated eggs. Most of the chicken will lead a normal life. One of the GA chicken lines develop granulosa cell tumours as adult and must be humanely euthanised before tumours progress to affect the animal's welfare. A second line of chickens have misshaped eyelids and must be monitored for eye infections and appropriately treated and managed if their eyes become infected. There is the consistent problem to breed homozygous animals for a genetic locus

while avoiding the health deficits associated with inbreeding of chicken.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild severity: 95% of animals
Moderate severity 5% of animals
What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The chicken is an important agricultural animal and it is important to study the genes involved in the fertility, disease resistance, and productivity traits of this animal for sustaining and improving food production. The biology of reproduction of birds is of interest in terms of understanding reproductive mechanisms of all vertebrate animals (including humans). The use of embryos and animals is necessary to study the interaction of the germ cells and somatic cells in the reproductive organs. The interaction of the germ cells and the somatic cells is what determines the fertility over the lifespan of the adult animal. The fertility needs to be measured by the formation of functional eggs and sperm in the sexually mature animal. The need to investigate gene function in chickens and the genes important for growth and development and the genes governing pathogen interactions necessitate the generation of novel animal models containing genetic modifications

To develop new methods to freeze chicken breeds from frozen material and demonstrate the re-creation of chicken breeds used in research, commercial and rare chicken breeds from frozen material necessitates the use of surrogate chicken hosts to carry the reproductive cells.
Which non-animal alternatives did you consider for use in this project?

We considered trying to grow chicken stem cells to generate the different cell types and tissues that could then be studied in cell culture. We could change the genes in these cells to study gene function.
Why were they not suitable?

The number of chicken cell lines is extremely limited and the ability to culture and change chicken stem cells into different tissues is lacking. Our skill in culturing chicken reproductive cells allows us to carry out some experiments in cell culture without using animals. Our use of chicken embryos mostly involves injecting germ cells at unregulated young stages in eggs and many of the experiments will also be performed before hatching of the chick. The use of the chicken as a model species is an improvement over using mammals. In this instance, the chicken offers advantages over using a mouse models as the mother does not need to be killed to obtain the embryo and a surgical manipulation is not needed to introduce donor reproductive cells into an embryo. The majority of experiments will also be carried out at unregulated developmental stages (before day 14.5 of incubation) Meiosis (halving of the chromosome number) occurs in the chicken embryo at day 16 of incubation so early meiosis can be studied in embryos before hatching.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For chicken, we have estimated the total number of genome edited and transgenic lines we will establish over 5 years, the number of genome edited offspring that will be bred during that time period. For each genome edited line, we will need to generate surrogate host birds that will carry the genetic material. For each experimental genome edited line, we use a power calculation to determine the number of animals we need to breed in order to carry out the required experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The cell culture of reproductive cells enables us to carry out some experiments without using animals. The donor reproductive cell injections are carried out at unregulated developmental stages in eggs and many of the experiments will also be performed before the chick would hatch. The use of avian species as models offers advantages over mammalian species for the study of embryonic development. In this instance, the chicken offers advantages over using a mouse models as the mother does not need to be culled to obtain the embryo and a surgical manipulation is not needed to introduce donor reproductive cells into the embryo. The majority of experiments will also be carried out at unregulated developmental stages (before day 14.5 of incubation) in the egg. Meiosis (halving of the chromosomal number) occurs in the chicken embryo at day 16 of incubation so early differentiation can be studied in embryos before they would hatch.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have worked to improve the efficiency of hatching surrogate chicks carrying the donor genome edited reproductive cells. We have added a protocol to investigate the incubation conditions to improve the hatch rate of chicks. We have and are developing genome edited surrogate hosts that lay 100% of the offspring from the donor genome edited cells. We have improved our genetic modification of the donor genetic material to screen for the genetic modification before the genetic material is introduced into the surrogate host chick embryos. We are also developing methods to reduce breeding by directly mating the surrogate hosts to generate pure offspring in a single generation which greatly reduces the number of chicken bred in these experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use the chicken as a model to study the genetics of poultry and birds and the ability to freeze eggs and sperm from bird species. The use of cultured avian reproductive cells that can be genetically modified to disrupt gene function and used to produce genome modified birds is unique to the chicken and avian species. The

access of the fertilised avian egg also means the surrogate host chicken can be hatched without killing the mother to obtain the egg. The transfer of reproductive donor cells into surrogate hosts is done in the laid chicken egg at young ages (unregulated stages) before the formation of a nervous system. The surrogate hosts chickens will grow normally but make eggs and sperm from another chicken breed or potentially another bird species.

Why can’t you use animals that are less sentient?

The chicken is an important agricultural animal and it is important to study the genetics of this animal for sustaining and improving food production. The understanding of the genes involved in the major production traits of disease resistance, feeding, and production traits will lead to more efficient agriculture. The biology of reproduction of birds is of interest in terms of understanding reproductive mechanisms of all vertebrate animals (including humans).

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have added a new protocol to investigate the incubation and manipulation of fertile eggs to increase our hatching rate from manipulated incubated eggs. We have instigated monitoring the weight of our newly hatched genome edited chicken to verify a normal growth curve for hatchlings.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All work is undertaken in close collaboration with our Named Veterinary Surgeons. Study protocol forms must be completed and submitted to them prior to proceeding with any experimental work and animal unit staff are closely involved in experimental work and will offer advice on improvements to experimental protocols and monitoring/raising of the animals.

The group contains all the competencies required to perform the majority of techniques and procedures outlined in this project. Where additional expertise is required, staff will undergo appropriate training until competent.

Animals will be kept in accordance with annex III of Directive 2010/63 EU and/ or Home Office Codes of Practice, as appropriate.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We communicate with our NVS on current developments to enrich the environment of housed chicken. Our poultry workers are trained in proper welfare techniques for birds and we are strictly monitored by our veterinary scientists and the Home Office on current practices. We visit the NC3R website for new information on animal use in research. We hold annual conferences veterinary ethics and welfare.

Home Office

NON-TECHNICAL SUMMARY

71. Glucocorticoids and Vitamin B3 metabolism

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Glucocorticoids, NAD+, Metabolism, Cushing's syndrome

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of the project is to investigate glucocorticoid interactions with nicotinamide adenine dinucleotide (NAD^+) pathways to determine their combined effect on energy metabolism and the underlying mechanisms that facilitate the potential relationship between the two.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Glucocorticoids are stress response hormones that are released to meet changing metabolic demands and as such are key regulators of energy metabolism and homeostasis. An excess of glucocorticoids, often seen in Cushing's syndrome lead to metabolic dysfunction and disease. The role of NAD^+ as a signalling substrate or as a metabolic cofactor in redox reactions is well understood, and like glucocorticoids altered content levels can lead to metabolic dysfunction. We and others have shown that using NAD^+ precursors to augment the NAD^+ metabolome can modify metabolism and positively impact metabolic function. Glucocorticoids interact with the NAD^+ metabolome and synthetic pathways, but the nature and impact of their combined interactions on metabolic function, as well as dysregulation, has not been studied. We propose that interrogation of this new axis of metabolic regulation will illuminate novel biology and therapies in a range of disease processes.

What outputs do you think you will see at the end of this project?

The research project will lead to a better understanding of the relationship between glucocorticoids and the NAD^+ metabolome as well as the mechanisms behind their interactions. This will include how they might directly affect each other or how they might alter other metabolic functions to indirectly affect the other. For example, this project will reveal whether glucocorticoid treatment directly alters the synthesis of NAD^+ or the total content of it or whether it might indirectly affect it through regulation of another metabolic function. Additionally, this project will reveal if increasing availability of NAD^+ can alter or rescue the effects of glucocorticoid excess. These planned treatments will enable the identification of the mechanisms that underpin any potential relationship.

Who or what will benefit from these outputs, and how?

All knowledge gained from the project will be shared with the scientific and clinical community and published in peer reviewed journals (output expected during the project).

In the short term (within the timespan of this licence) knowledge of the mechanisms behind the relationship between glucocorticoids and the NAD^+ metabolome and their combined effect on energy metabolism and homeostasis will become known. This will include knowledge of how glucocorticoids might affect the NAD metabolome and alternatively whether NAD^+ might be able to help combat glucocorticoid excess. This knowledge will then be used to inform human experiments (beyond 5 years) that will test if the findings of this project translate into humans to combat issues caused by glucocorticoid excess.

How will you look to maximise the outputs of this work?

Discoveries as well as negative results will be shared with the wider scientific and clinical communities through presentation at national and international conferences and publication in highly ranked peer reviewed journals. We will also share best practice of new techniques and newly developed protocols with collaborators. We will engage with the public through events organised by the establishment to showcase our research.

Species and numbers of animals expected to be used

- Mice: 2400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The aim of animal studies in this project is to examine the relationship between glucocorticoids and the NAD^+ metabolome in order to understand their shared impact on energy metabolism and homeostasis. Mouse genetics and genome manipulation is uniquely advanced and there is a wealth of understanding of murine physiology making mice a most tractable mammalian model. The broad range of reagents and testing kits suitable for murine work makes them more suitable than other rodent models.
Young adult mice (3-6 months) were chosen to avoid developmental issues that can be seen when treating younger mice (<10 weeks) with glucocorticoids.

Typically, what will be done to an animal used in your project?

Animals will be treated with glucocorticoids to induce models of glucocorticoid excess which will lead to the development of a cushingoid phenotype. This phenotype will be characterised by an increase in adiposity, a reduction in muscle size, a reduced rate of weight gain or even weight loss. This model will be used to test the effect of glucocorticoids on the NAD^+ metabolome. Some mice will be treated with vitamin B3 (NAD^+) precursors during or after the development of this phenotype to test the therapeutic effect of NAD^+ in this model, as well as its effect on glucocorticoid metabolism. If not treated with glucocorticoids or NAD^+ (vitamin B3) precursors animals will instead be given a vehicle control or sham injection. Furthermore, the effect of glucocorticoids and vitamin B3 precursors on glucose homeostasis and energy metabolism will be investigated. Treatments will not exceed 4 weeks apart from vitamin B3 precursor supplementation in the drinking water. Mice will be humanely culled at the end of each experiment allowing for tissue collection and blood samples to be taken.

What are the expected impacts and/or adverse effects for the animals during your project?

Glucocorticoids are naturally occurring hormones, while they are not toxic, excess can lead to Cushing's syndrome which causes increased adiposity, muscle atrophy, hypertension and insulin resistance. Glucocorticoid treatment doses and duration in our project are carefully chosen to induce a cushingoid phenotype while minimising adverse effects for the animals. While animals will experience an asymptomatic muscle atrophy, it is not anticipated that animals will experience pain or discomfort from glucocorticoid treatment, other than the transient discomfort from subcutaneous injections. Body weight and body condition will be closely monitored to detect any deterioration in muscle mass as soon as possible.
Mice treated with just vitamin B3 precursors are unlikely to experience any adverse side effects as a large body of research already exists to support their use for increasing NAD^+, these compounds are well tolerated and have minimal effects on welfare, and in general promote metabolic health.
Glucose and insulin tolerance testing lead to transient pain during blood sampling (2min) when the initial tail incision is made and when subsequent blood samples are taken from the same incision over a period of up to 3

hours. To ameliorate this, a local anaesthetic will be applied before venesection. If tolerance measurements are repeated, mice will be resting for 2 weeks between measurements. For energy assessment in the TSE Phenomaster System mice have to adapt to the new environment, which might lead to slight weight loss in some animals. To ameliorate this an acclimatisation phase allows the mice to get used to the environment in their original litter groups before they are single housed during the isolation phase. The energy assessment itself is performed by measuring O_2 and CO_2 concentrations non-invasively while the mice live in their cages. Mice will rest between TSE measurements and tolerance testing for a week.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

About 80% of mice will undergo a combination of procedures, like glucocorticoid treatment, glucose and insulin tolerance testing and energy assessment or vitamin B3 precursor injection followed by glucose and insulin tolerance testing or mice will undergo glucose, insulin tolerance testing. This will be classed as moderate severity.
The remaining 20% of mice will not undergo a combination of procedures and will be classified as mild.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We use non-animal methods to identify and validate the vitamin B3 precursors that can be used to influence glucocorticoid metabolism in cells. While these are important studies, these conditions are artificial and cannot give an accurate picture of glucocorticoids effects on different organs and the whole body in mice or humans. Glucocorticoids are produced in the adrenal cortex, but effect many different organs like muscle, liver and adipose tissue. Thus, we use mouse models to study how glucocorticoids and vitamin B3 precursors can influence energy and glucose metabolism in different organs.

Furthermore, in mouse models specific genes of the bio synthetic pathway from vitamin B3 precursors to NAD^+ can be inactivated to allow to examine their effect of biochemical pathways *in vivo*.

However, continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace *in vivo* animal use.

Which non-animal alternatives did you consider for use in this project?

In vitro data obtained from murine cell culture approaches has guided the proposed *in vivo* studies. We always conduct cell culture based experiments to justify the need to use animals.

Why were they not suitable?

Not all questions can be addressed in cell culture, there are no immortal cell lines available to study the complex interactions of glucocorticoid hormones with different organs. Organoid cultures are still technically challenging.

Reduction

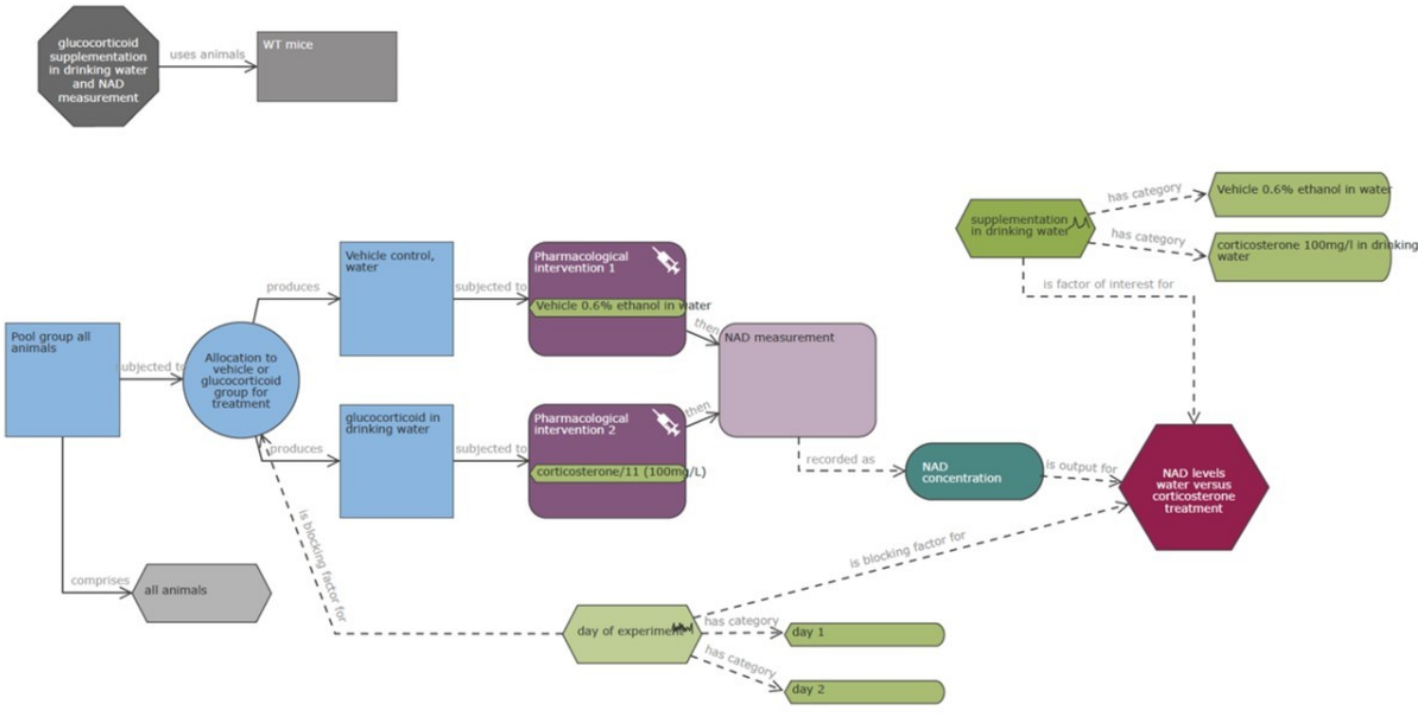
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

When possible, we will always use power calculations to determine the number of animals that should be used. For most quantitative experiments, animal cohort size will be calculated via power analysis. Expected effect size will be determined through consultation of the literature, cell culture based *in vitro* analysis or through small pilot experiments when possible.
We have used statistical methods to calculate how many animals we need to get meaningful data, depending on the type of glucocorticoid used for treatment and the outcome measures of the NAD^+ metabolome we will look at. We will carefully consider which treatments and outcome measures will be tested sequentially, thus reducing the number of animals being used.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used the NC3Rs' Experimental Design Assistant to design experiments (PROTOCOL 1,example) and taken advice from researchers who have performed similar research projects in the past.



We aim to reduce animal numbers, methods to reduce subjective bias, and appropriate statistical analysis

without compromising the scientific objectives. When possible, experiments will involve a factorial design that will maximise the information obtained from a minimal number of animals. Furthermore, non-invasive energy metabolism assessment and subsequent glucose or insulin tolerance testing can be performed on the same animal over a period of time, thus reducing the number of animals used. As importantly, at the end of all *in vivo* experiments, several tissues will be isolated and a large proportion of the scientific output in terms of NAD metabolism will be generated from these tissues in further *in vitro* experiments for example primary cell culture, protein analysis, metabolomics and genetic studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies to estimate variability and perform power calculations to calculate sample sizes.
Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.
Even the most effective breeding strategies lead to some wastage of non-target animals. This can be avoided in our studies as we will be using WT animals to study physiology. If we are unable to estimate an effect size from our *in vitro* data, the literature, or our collaborators, we will conduct small pilot experiments.

All tissue surplus to requirement will be stored and made available to collaborators within the institution and beyond.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use WT mice to study how glucocorticoids effect the NAD^+ metabolome and how altered NAD^+ bioavailability (via vitamin B3 precursors) effects glucocorticoid activity.
Glucocorticoid treatment doses and duration in our project are carefully chosen to induce a cushingoid phenotype while minimising adverse effects for the animals. While animals will experience an asymptomatic muscle atrophy, it is not anticipated that animals will experience pain or discomfort from glucocorticoid treatment, other than the transient discomfort from subcutaneous injections if they are chosen as the appropriate route of administration. If possible administration in the drinking water will be used as a more refined route of glucocorticoid administration. Body weight and body condition will be closely monitored to detect any deterioration in muscle mass as soon as possible. Young adult mice (36 months) will be used to avoid developmental issues that can be seen when treating younger mice (<10 weeks) with glucocorticoids.
Even though vitamin B3 precursors are well tolerated and usually have no adverse effects, we will use pilot studies to help us to develop dosing regimens for vitamin B3 precursors if administration is not oral to further minimise risk of adverse effects.
To measure the energy metabolism of mice *in vivo* we employ the TSE Phenomaster System. While the mice live in their home cages, CO_2 production, O_2 consumption as well as food and drink intake can be monitored without any disturbance to the animals.
Other methods employed are considered the gold standard (ipGTT, ipITT, ipPTT) and as such are well

understood and practised, helping to minimise the risk and distress to the animal. These methods are designed to collect the minimal blood volumes required for glucose measurements, which again minimises both the risk and distress to the animal.

Why can't you use animals that are less sentient?

Mice are the least sentient species available to achieve our objectives. Using mice allows us to explore the relationship between glucocorticoids and the NAD^+ metabolome in a physiologically representative model, to produce data that can greatly inform treatments or further investigation within humans. Mice and humans share 97.5% of their DNA sequences, making mice preferential to using less sentient species such as zebra fish or drosophila.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Discussion with academics both internally and externally, as well as extensive review of the literature, ensures we are aware of, and continue to develop refined treatment doses and administration techniques. This combined with prior *in vitro* work ensures we have a strong understanding of the maximum tolerated dose, the minimum effective dose, and sub-optimal doses. This minimises the chances of an animal experiencing excessive adverse side effects.
Animal welfare is a key consideration in all of our protocols, and we will be guided by our NACWO and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. Following every experiment and regularly during group meetings we will review our procedures from a welfare standpoint to identify any potential for refinement.
We are fortunate to have excellent colleagues both in academic groups and within our animal facility with extensive, relevant animal procedure experience, from whom we can learn refined techniques. Our team will undergo extensive training on dead animals and require to be authorised by our animal facility before being allowed to perform a procedure on live mice.

Examples: The TSE phenomaster System allows to quantify energy metabolism by measuring O_2 and CO_2 concentrations, food and drink intake while the animal is living in an IVC cage without any disturbance. For glucose and insulin tolerance testing a single incision will be made on the tail to take small sequential blood samples rather than sequential cutting for blood sampling from tail vein. Cardboard tubes are used for refined handling and body condition charts for humane endpoints. Whenever scientifically possible the most refined route of administration will be used for vitamin B3 and glucocorticoids. This means administration in the drinking water will be the preferred to injection. To establish suitable doses and duration of vitamin B3 administration small pilot studies will be used, before larger more informative will be conducted.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will constantly consult the literature for experimental design, best practice, and humane endpoints for metabolic and diabetes research in animals, and we will publish in accordance with ARRIVE guidelines published by the NC3R's.

We will consult Simon Bate's book, The design and statistical analysis of animal experiments, for experimental design, statistical analysis, and sample size calculations.
Prior to all experiments we will also consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment (PREPARE: guidelines for planning animal research and testing. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.PMID: 28771074).

The LASA giudlines: RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)
Jones HRP, Oates J, Trussel l BA (1999) An applied approach to assessment of severity. In: Humane End points in Animal Experiments for Biomedical Research (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 40±7

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will perform literature searches, attend vendor's information sessions, seminars and conferences to find out about new technology and new approaches that we could implement.
We will comply with the ARRIVE guidelines 2.0 (Animal Research: Reporting In Vivo Experiments; www.nc3rs.org.uk/arrive), a NC3Rs-developed checklist of the essential information that should be included in publications reporting animal research.
My Lab group twitter follows the NC3Rs and lab members are subscribed to the NC3Rs newsletter - helping us keep up with advances in the 3Rs which will be discussed and implemented through our lab meetings.

Home Office

NON-TECHNICAL SUMMARY

72. Hormone and genetic regulation of skeletal tissue maintenance and repair

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Osteoporosis, Osteoarthritis, Fracture, Thyroid hormones

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The project has two main aims:

1. To determine the role of thyroid hormones and their mechanisms of action in bone and joint tissue maintenance, injury and repair.

2. To identify the cellular and molecular mechanisms of disease onset and progression in osteoporosis and osteoarthritis.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Half of all adults are affected by bone and cartilage disorders. The two most common musculoskeletal disorders are osteoporosis and osteoarthritis.

Osteoporosis is a chronic condition characterised by loss of bone tissue and a decrease in its mineral or calcium content, resulting in decreased bone strength and an increased risk of fracture. Living with osteoporosis means that individuals are at a higher risk of breaking or fracturing a bone following a minor injury or a fall. The lifetime incidence of fracture for a 50-year-old in the UK is 40% for women and 13% for men, and osteoporosis already costs the NHS more than £1.7 billion per annum.

Osteoarthritis is a chronic condition that can affect any of the joints, but commonly involves weight bearing joints such as the hips or knees. It results from prolonged wear to the cartilage lining of the joint and when this happens all the tissues within the joint become more active than normal as the body tries to repair the damage. The repair processes may change the structure of the joint, but will often allow it to work normally and without any pain and stiffness. However, the repair processes don't always work so well and progressive changes to the joint structure can cause or contribute to pain, swelling or increasing difficulty with mobility. Osteoarthritis affects 15% of the population and its cost is estimated at 1% of gross national product. This financial burden will inevitably increase as our population ages and disease incidence rises.

The few available treatments for osteoporosis generally prevent bone loss but their effectiveness is limited by side effects and patient acceptability, whilst there are no effective drugs that prevent osteoarthritis or delay its onset and progression. Thus, there is urgent need to advance understanding of the mechanisms of bone and cartilage formation and repair in order to develop new treatments.

The basic cellular and molecular programmes that regulate development of bone and joints are also essential for tissue maintenance and its response to injury and chronic disease. Hormonal regulation of these processes is important. Osteoporosis and osteoarthritis each affect men and women to differing degrees but the mechanisms responsible for these sex differences are unknown. Exposure to thyroid hormone excess, due to thyrotoxicosis (an immune condition in which the thyroid gland produces and secretes too much hormone into the circulation) or following excessive thyroid hormone replacement in patients with hypothyroidism (deficiency of thyroid hormone in the circulation), is an important risk factor for osteoporosis and fracture. Furthermore, the local supply of thyroid hormones in the joint has been associated with susceptibility to osteoarthritis in large population studies, but it remains unknown how thyroid hormone actions in joint tissues modify disease risk.

The work in this project will identify new genes and pathways that underlie development of chronic tissue damage in the skeleton and will investigate how hormones, such as thyroid hormone, regulate these processes. This work will provide essential new understanding into the origins and development of skeletal diseases and will help to uncover new strategies for drug development in osteoporosis and osteoarthritis.

What outputs do you think you will see at the end of this project?

New knowledge

This work will provide a detailed understanding of the fundamental role of thyroid hormones in the maintenance and repair of bone and joint tissues. This new information is particularly applicable to the role of hormones in osteoporosis, fracture repair and osteoarthritis. The primary expected benefits will include publication of new scientific knowledge regarding the individual cell types in bone and joints that respond to thyroid hormones and the signalling pathways that are activated or repressed by hormone stimulation of these cells. The work will also identify new genes that specify bone and joint structure and function and new molecules and signalling pathways that regulate skeletal development, bone maintenance, cartilage degeneration and fracture repair. Publication of these findings will provide the research community with fundamental new knowledge, particularly relating to the identification of specific proteins that could be developed for measurement in the blood as new markers of disease, or which could be used to identify and understand the actions of new drugs. Such advances will ultimately help in the diagnosis, prevention and treatment of skeletal diseases such as osteoporosis and osteoarthritis.

New resources

A further benefit of this work will be the generation of new and specific genetically altered mice that will be made available to scientists working in the musculoskeletal and other fields. Furthermore, tissue samples from disease models used in these studies will be archived and stored so they can be provided to other scientists and collaborators for their studies.

Datasets

The data describing the characteristics of the joint in osteoarthritis, which are generated in these studies, are being made freely available for the benefit of the scientific community via the International Mouse Phenotyping Consortium website. Furthermore, datasets containing functional information on the genes that control joint characteristics will be uploaded onto the International Federation of Musculoskeletal Research Societies (IFMRS) Musculoskeletal Knowledge Portal, which is a new open-access integrated data mining platform aimed at accelerating discoveries for musculoskeletal traits and diseases.

Who or what will benefit from these outputs, and how?

Throughout the life of this project, data produced will be presented at national and international conferences and published in academic journals. The new information will provide a fundamental understanding of how hormones and newly identified disease susceptibility genes regulate cellular and tissue responses to chronic damage and injury in bone and joints. Protocols and methodological refinements or examples of best practice that we identify during this project will be published in scientific journals wherever possible and are being made available via the International Mouse
Phenotyping Consortium open-access website. We will archive and make post-mortem tissue available to collaborators and other scientists working in musculoskeletal research or other fields.
In the medium term the pharmaceutical industry will be interested in new knowledge that will help to understand the mechanisms of action of new drugs, which could be used to treat musculoskeletal diseases.
The long-term potential benefits of this study are that data generated may have far-reaching implications for the treatment of osteoporosis and osteoarthritis, benefitting patients and clinicians by contributing to the development of novel drugs that will ultimately reduce the economic and health burden caused by these common and chronic diseases.

How will you look to maximise the outputs of this work?

Findings from this work will be made available to other scientists through publication in open-access journals, presentations at scientific conferences and meetings, and via open-access websites to assist the research community. These routes of dissemination have already fostered collaborations outside the musculoskeletal

research arena, and we anticipate that the new knowledge gained in these studies will encourage further broad collaborations. New disease models and other transgenic mice developed in these studies, post-mortem tissue samples, and phenotype and genetic datasets will be valuable and made available to other scientists interested in therapeutic targeting and drug development for osteoporosis, fracture healing and osteoarthritis.

Species and numbers of animals expected to be used

- Mice: 10000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

It is critical to study disease mechanisms in an animal model that resembles the human condition as closely as possible. The mouse is the least sentient species, which can be used to obtain meaningful results that will both advance scientific understanding and be applicable to human disease. Many physiological processes in humans are the same and maintained in mice. Thus, skeletal disorders result from abnormalities that may occur during intra-uterine development, post-natal growth or adulthood in both species, whilst structural properties of bone and joints depend on movement and weight bearing. Furthermore, the same genes, hormones and regulatory factors that control; (i) bone and joint development and maintenance, (ii) the onset and progression of disease in osteoporosis and osteoarthritis, and (iii) the healing processes involved in fracture repair are also identical and maintained in both mice and humans.
Overall, mice are the most appropriate species for these studies as they allow developmental and temporal aspects of the mechanisms of skeletal disorders to be investigated using well established disease models.

Typically, what will be done to an animal used in your project?

Mice will undergo procedures to manipulate their hormonal status by giving hormones or inhibitors in the drinking water or, in some cases, by surgery to remove the ovaries and mimic the menopause. To study the effect of altered hormone responses in the skeleton, animals will also undergo surgery to provoke the onset of mild arthritis of the knee or create an internally splinted and stable fracture. Surgical procedures are shorter than 30 minutes in all cases, performed under aseptic conditions and animals are fully anaesthetised. Mice are monitored very closely and recover fully within a few minutes after surgery. They only experience a mild degree of lameness for a short period after procedures to provoke the onset of mild arthritis or generate a stable fracture, and this does not prevent free movement or normal eating and drinking. All animals routinely receive painkillers to prevent discomfort and they are housed for up to a maximum of 16 weeks with appropriate bedding, nesting material and cage toys within an enriched environment. At the end of the study animals will be killed humanely and their bone and joint tissues collected for analysis of samples using X-rays, microscopes and molecular techniques.

What are the expected impacts and/or adverse effects for the animals during your project?

Mice will receive hormones or inhibitors of hormone production in their drinking water over several weeks to produce either thyroid hormone excess or deficiency. Thyroid hormone excess can result in increased activity,

weight loss despite good appetite and muscular weakness, whilst marked thyroid hormone deficiency may cause lethargy and weight gain. Such symptoms occur in severe and established chronic hormone disturbances and, in our experience, they are very unlikely to occur with the protocols used in these studies (less than 1% of mice develop problems). Nevertheless, animals will be monitored closely, examined at least every 5 days and weighed every week to ensure they do not experience any distress.
Some genetically altered mice may be given a hormone treatment by mouth in order to produce the genetic alteration in a single cell type within the skeleton but not in other parts of the body. The reason for doing this is to investigate the role of hormones in skeletal tissues without affecting their function in other organs or in the circulation. Such genetic alterations are mild and have no harmful
consequences. Nevertheless, tamoxifen treatment can rarely cause local inflammation, loss of appetite and hair loss, and may result in still births if given to pregnant dams. In our experience problems are highly unlikely as we have previously performed pilot studies to determine the safe effective dose and method of drug delivery that result in minimal side effects. Adverse reactions resulting in mortality due to tamoxifen administration can occur very rarely in less than 1% of animals. All animals will be checked and monitored for minor physical changes and signs of distress at least daily and will be weighed regularly.

Mice may also undergo minor surgery to either (i) remove the ovaries and mimic the menopause, (ii) provoke mild arthritis of the knee or (iii) create an internally splinted and stable fracture in the lower leg. After removal of the ovaries, mice will be given painkillers and post-operative care just like people receiving treatment in hospital and they recover quickly. Following arthritis or fracture surgery, mice are fully mobile and weight bear normally on the operated leg within a few minutes of recovery. Mild lameness can occur immediately after surgery in less that 10% of animals, but this does not prevent free movement and normal eating and it resolves rapidly. Nevertheless, all mice will be monitored carefully and given painkillers for an extended period of up to 3-4 days post-operatively if necessary. Some animals can scratch or pull sutures apart before the wound has healed and if this happens wounds will be cleaned are re-closed. Most animals have an uneventful recovery and will be housed in groups to encourage social integration. Together with regular gentle handling and cage enrichment these measures minimise distress and alleviate boredom.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

There are eight protocols in this licence.
The first four protocols include the procedures that are used to generate genetically altered animals; two of these protocols are of mild severity (we expect 5% of mice to experience mild severity and 95% to experience sub-threshold severity in these protocols) and two are of moderate severity (we expect 5% of mice to experience moderate severity and 95% to experience mild severity in these protocols).
The fifth protocol is used for the breeding and maintenance of genetically altered animals and is of mild severity (we expect 75% of mice to experience mild severity and 25% to experience sub-threshold severity in this protocol).
The sixth protocol includes the procedures that are used to alter hormone levels in mice and is of moderate severity (we expect 45% of mice to experience moderate severity and 55% to experience mild severity in this protocol).
The seventh protocol includes the procedure that is used to provoke osteoarthritis and is of moderate severity (we expect 100% of mice to experience moderate severity in this protocol).
Finally, the eighth protocol includes the procedure that is used to produce a stable fracture of the shin bone and is of moderate severity (we expect 100% of mice to experience moderate severity in this protocol).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of animals is essential for these investigations as other options that were considered in detail and discussed below are not able to deliver what is needed. Hormones and other regulatory factors act in the microenvironment of the skeleton and the bone marrow to control bone development and maintenance. Bone and joint diseases may result from abnormalities that arise during fetal development, post-natal growth or in adulthood, whilst maintenance of bone structure and strength is dependent on movement and weight bearing. Animals are similarly essential for studies of the onset and progression of osteoarthritis and fracture healing. These dynamic processes involve multiple cell types, recruitment of inflammatory cells from the circulation, formation of new blood vessels, and migration of bone-forming cells in order to maintain and repair the sites of injury. All these intricacies cannot be modelled ex vivo, and investigation of factors that initiate and modify disease progression must therefore involve whole animals.

Which non-animal alternatives did you consider for use in this project?

We contribute to and make extensive use of the Musculoskeletal Knowledge Portal (http://mskkp.org), which is curated by the International Federation of Musculoskeletal Research Societies (IFMRS) and contains genetic and gene function databases that aim to accelerate discoveries for musculoskeletal traits and diseases. Thus, we have access to cutting-edge information from genetic, studies in populations, bone cell culture and gene function studies in humans and several species of animal model. Computer modelling and bioinformatic analysis of these datasets enable us to focus our animal experiments to investigate the complex physiological regulation of gene functions in the skeleton using the whole organism and ensure our studies are relevant to human disease. It further allows us to test molecular mechanisms of hormone action and genetic control of skeletal cells in vitro, using primary cell cultures of bone forming osteoblasts, bone resorbing osteoclasts and cartilage forming chondrocytes.

Why were they not suitable?

The computer modelling, bioinformatics and cell culture models that we employ to investigate molecular mechanisms that underpin bone and joint abnormalities and diseases are extremely important and useful. They complement studies in live animals and give an overall much greater understanding of the onset and progression of skeletal disorders. On their own, however, they cannot provide a comprehensive understanding of skeletal tissue maintenance and repair in response to chronic disease or injury, and so animal studies remain essential.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals to be used was determined using published information, previous experience and the biological variation encountered during analysis of bone and joint parameters relating to structure and strength. Our calculations account for different time points of study to investigate the onset and progression of disease, the use of complementary analytical methods to ensure robust outcome measures and give us assurance that our experimental approaches will detect biologically meaningful effects when they are present.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We follow the international PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines, have taken advice from statisticians and have planned the studies to enable them to be published according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, which are intended to improve the reporting of research using animals and have been approved by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). Furthermore, we have developed a tissue collection and storage strategy that enables us to maximise the use of tissue by employing sequential and complementary analytical techniques on the same sample in order to gain the most information possible from a single animal. This minimises animal numbers and prevents experimental duplication.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Primary bone cell culture will be used in place of animals to investigate molecular mechanisms wherever possible. Studies will include hormone manipulations to investigate cell maturation and examine gene regulatory responses. Data from these in vitro studies, and analyses of publicly available musculoskeletal datasets (http://mskkp.org), will be used to inform in vivo approaches and pilot studies. This strategy will provide preliminary data that will enable us to ensure the design of animal studies will be statistically robust and informative, relevant to human disease, and efficient. We have also developed an efficient breeding strategy to minimise animal numbers by using an osteoarthritis model only suitable in males, together with a fracture-healing model that is independent of gender and will be performed in females. This ensures all mice that are bred and maintained will be informative and can be used for experiments whenever possible.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use the least traumatic and most refined disease models; ensuring best practice by adhering to the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines; and ensuring the best possible animal husbandry is adopted according to LASA guidelines.
The osteoarthritis, stable tibia fracture and osteoporosis provocation (ovariectomy) models were established and validated in mice with predictable outcomes. They are the least traumatic of the published provocation models and represent the gold standard in the field, being internationally recognised by the Osteoarthritis Research Societies International (OARSI) and American Society for Bone and Mineral Research (ASBMR). We ensure that scientists working with animals have gained the highest surgical expertise, use the most-refined and adhere

to up-to-date aseptic techniques, and incorporate appropriate anaesthetic and adequate analgesia to minimise pain and facilitate rapid recovery and restoration of full-mobility following surgery.
In studies to manipulate thyroid status we adhere to the published “American Thyroid Association guide to investigating thyroid hormone economy and action in rodent and cell models” that contain the most up-to-date refinements for investigation of thyroid hormone action in rodent models.

Why can’t you use animals that are less sentient?

The mouse represents the least sentient species that can be used to model human musculoskeletal disease. Conserved mechanisms of skeletal development and skeletal regulation are well documented in mice and humans, and studies in mice have already led to the development of new drugs for the treatment of osteoporosis. Our studies, in collaboration with human geneticists, have established that genes associated with increased risk of osteoporosis and osteoarthritis in humans share similar functions between species, and we have shown that genetic alteration in the mouse faithfully reproduces human disease. In order to study disease onset and progression and understand the underlying pathological mechanisms, it is essential to study developing, juvenile and adult animals. Mice are the most appropriate species because genetic manipulation is well-established. Furthermore, many genetically altered strains are already available and validated and can be used by others without the need to generate them again.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have noted and incorporated a number of refinements to each of our protocols since we started to use mouse models over 25 years ago. We continue to monitor animals closely, and with the Veterinary Surgeon constantly assess possible improvements in husbandry, postoperative care, pain management, and animal environments.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow international PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines, plan and conduct studies according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and use National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) guidelines to ensure our animal experiments are as robust and reproducible as possible. We follow research conduct, protocol and reporting guidelines from the Osteoarthritis Research Societies International (OARSI), American Society for Bone and Mineral Research (ASBMR) and American Thyroid Association (ATA) to ensure our studies on hormone regulation of the skeleton and joints are the most refined, up-to-date and relevant to understanding the mechanisms that underpin human musculoskeletal disease.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We read the monthly updates from the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) on their events and publications and their e-Learning resources. All of these detail advances in replacement, refinement and reduction techniques and best practice and provide information how to put these in place. Furthermore, I have extensive experience as member and chair of Animal Welfare Ethical Review Bodies, ensuring I am fully up-to-date and conversant with the principles and practices of the 3Rs.

Home Office

NON-TECHNICAL SUMMARY

73. Host-parasite dynamics in spatially structured host populations

Project duration

5 years 0 months
Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
voles	juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The work focuses on examining the impact of host spatial structure on parasite dynamics and diversity.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Environmental change can increase the likelihood that diseases will transmit from wild animals to livestock or humans. By influencing the number and distribution of wild animals, land-use modification and the increasing fragmentation of natural habitats can have important impacts on the prevalence of different diseases. Such changes can also lead to the emergence of pathogen strains better suited to the new conditions. To understand these issues, we need information on the prevalence and strain diversity of pathogens infecting wild animal populations that inhabit fragmented natural habitats, as well as to what extent pathogens are shared between co-existing animal species.

What outputs do you think you will see at the end of this project?

By comparing the prevalence and diversity of different pathogen species and strains, we will achieve a better fundamental understanding of disease dynamics in fragmented wildlife populations. Our work will shed light on the relative importance of factors such as the ability to infect multiple wildlife species and infection length. Ultimately, this research will hopefully benefit the health of livestock and people by improving our understanding of how the increasing fragmentation of natural habitats may influence the risk posed by diseases that can be transmitted from wildlife populations

In addition, this research will also have direct benefits for water vole conservation. The species has suffered the largest decline of any British mammal in the last century, largely due to predation by feral American mink. One approach to the conservation of the water vole is the reintroduction and translocation of animals to form new populations. The research will provide further information for reintroduction and translocation programmes on (i) the parasites that can infect water voles and the other wildlife species that may transmit infections to water voles, (ii) how water voles move across landscapes between populations and (iii) the likely threat of disease spread between adjacent reintroduced populations.

Who or what will benefit from these outputs, and how?

At this stage, the principle beneficiaries will be other researchers working in the fields of disease ecology and pathogen evolution. Research findings will be disseminated to the research community through published articles and presentations at conferences.

In addition, we will use our close links with conservation practitioners involved in water vole translocation projects to ensure relevant information is effectively shared.

How will you look to maximise the outputs of this work?

In addition to primary scientific publications and conference presentations, we will seek to make the data available to other researchers. Our research team has a long history of collaboration with theoreticians and statisticians to ensure maximum gain from the datasets generated. For example, teams from Australia, France and the UK have used our wild rodent data to develop and test approaches and methods for analysing “real” disease data.

Species and numbers of animals expected to be used

- Other rodents: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We want to understand disease in natural, wild populations. Wild rodents are an ideal model system for this as they exhibit a range of population structures (e.g. large, relatively continuous populations; small, fragmented populations), are easy to catch, and are infected with a range of diseases. Sampling will include a range of life-stages. Animals are not caught in traps until they are weaned, and capture of animals < 1month old, heavily pregnant or > 12 months old is uncommon (<5%). It is important to sample a range of ages so that the relationship between age and infection status, and age and movement rates can be determined.
Typically, what will be done to an animal used in your project?

Animals will be caught in traps. They will be marked using appropriate methods for future individual identification (e.g. tags inserted under the skin, small ear biopsy). They will have a small blood sample collected from the end of the tail. Animals will typically be handled for <10 minutes before being released back into the wild at the place of initial capture.
What are the expected impacts and/or adverse effects for the animals during your project?

These procedures are not expected to result in more than momentary pain or distress and cause no lasting harm. The stresses placed on individual animals due to handling are minimised through good training and efficient field technique. Animals are expected to fully recover from the procedures within a few minutes.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild (100%)
What will happen to animals at the end of this project?

- Set free
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of the work is to understand host-parasite dynamics in natural populations - hence the programme consists of observational studies, and must be based on the study of whole animals in their natural environment.

Which non-animal alternatives did you consider for use in this project?

Simulation modelling.
Why were they not suitable?

Simulation modelling will be used to understand likely disease dynamics under different scenarios. However,

ultimately these need to be tested with the field data, and therefore they are not sufficient to address the project objectives by themselves.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers are based on our previous experience of rodent numbers in our study system, and are sufficient to detect key effects linked to our objectives (e.g. effect of the number of animals on infection rates).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To maximise the information that can be obtained from the studies, we will use advanced statistics to ensure that the natural variability in the data collected during studies of natural populations is properly accounted for. This helps ensure objectives can be met with fewer animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Each year specific objectives will be determined for the field season. Annual analyses of data will enable targeting of population sampling based on these objectives. For example, targeting sampling at large populations or populations that have naturally recolonised empty areas of habitat.

When expanding the studies to include additional populations we will conduct preliminary trapping prior to conducting any licensed procedures to ensure that sites with appropriate mixes of wildlife species are selected.

As the same samples are used to test for many diseases, overall this programme of research will use fewer animals than a similar research programme that focussed on one disease.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The water vole is probably the best available system for such a study due to the range of population structures that occur. The other species that will be sampled are the species that are most likely to share parasites and diseases with the water vole. Methods include inserting tags under the skin of animals, fur-clipping or taking an ear-punch for individual identification and taking a small blood sample from the animal for detection of

pathogens. All of the species can be easily handled, facilitating sample collection and minimising any distress.

Why can’t you use animals that are less sentient?

Sampling will include a range of life-stages. Animals are not caught in traps until they are weaned, and capture of animals < 1month old, heavily pregnant or > 12 months old is uncommon (<5%). It is important to sample a range of ages so that the relationship between age and infection status and age and dispersal rate can be determined.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The stresses placed on individual animals are minimised through good training and efficient field technique. This is essential for both ethical reasons and to ensure we have as little impact as possible on the natural processes under study. Trapping will always be performed by a competent person using a method which does not cause the animals avoidable pain, suffering, distress or lasting harm. Traps are calibrated to minimise the risk of catching smaller, non-target species and only baited with food attractive to the target species. Traps are regularly checked.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

2016 Guidelines of the American Society of Mammologists for the use of wild mammals in research and education

Journal of Mammalogy, Volume 97, Issue 3, 9 June 2016, Pages 663–688,

https://doi.org/10.1093/jmammal/gyw078 http://mammalogy.org/uploads/committee_files/CurrentGuidelines.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Attend annual Licence holder refresher meetings, keep up to date with literature in field and collaborate with others in the same field.

Home Office

NON-TECHNICAL SUMMARY

74. How does metabolism affect the immune response in health and disease?

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Immunity, Autoimmunity, Lymphoma, Cancer, Metabolism

Animal types	Life stages
Mice	juvenile, adult, pregnant, neonate, embryo, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We seek to understand how changes in metabolism can affect the immune system in health, autoimmunity, and cancer.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our current understanding of the role of the immune system in causing autoimmune disease and in responding to cancer is very incomplete. This project will examine the effects of metabolism on the normal and abnormal immune systems, with the overall aim of identifying pathways which might represent novel therapeutic targets. This is important both to advance our knowledge of fundamental science in immunology, but also to guide new approaches to treatment, which are at present limited in efficacy and subject to significant toxicities. The idea that metabolic pathways might control the immune response has emerged as a result of *in vitro* investigation, but in order to determine if there is fidelity to normal physiology, both to inform science and to develop therapeutics, it is necessary to explore these pathways and their modification *in vivo*, in relevant and important disease models.

What outputs do you think you will see at the end of this project?

Key areas this project will examine include:

- The role of metabolic pathways (in particular amino acid synthesis and metabolism and mitochondrial metabolism) in the germinal centre reaction, and protective immunity in general (against infection and cancer)
    - *This work will uncover how immune cell metabolism influences the formation of protective antibodies which bind to their target with high affinity, and the development of immune memory, which occur in a process known as the germinal centre reaction (GC)*
- Whether cellular metabolic programmes determine progression from normal GC B cells to lymphoma
    - *This objective will help to understand if the specific metabolism B cell use in the GC reaction helps program the lymphoma cells which arise when this process goes wrong.*
- The metabolic dependencies of lymphoma *in vivo*
    - *This aim will help to understand if lymphoma cells have specific metabolic adaptations in vivo which can be targeted for therapy. Almost all previous work on lymphoma metabolism has been in cell culture, which has poor fidelity to in vivo and therefore poor translational potential.*
- How alteration of cell-intrinsic metabolism affects the function of regulatory T cells
    - *How regulatory T cells, which are essential for avoidance of intestinal inflammation in response to the microbiome, utilise metabolites is not clear. This aim will genetically alter these cells to understand the importance of key metabolic genes.*
- How autoimmunity (lupus, inflammatory bowel disease, and arthritis) affects immune cell metabolism
    - *This objective will identify how metabolism is deranged in autoimmune disease, providing new information about the drivers of pathology and revealing potential therapeutic targets.*

- The effect of modification of external metabolites through alteration of the diet on immune cell metabolism
    - *Very little is known about how changing the availability of nutrients or metabolites in the environment affects the metabolic phenotype of immune cells. In this aim, we will uncover the effect his has on the cellular level.*

This project will lead to new scientific knowledge, including the potential identification of novel therapeutic targets. The primary output will be peer-reviewed scientific papers, presentations at scientific conferences, and through a peer reviewed public engagement outlined in the supporting grants.

Who or what will benefit from these outputs, and how?

The scientific community will benefit from the experimental results of this work immediately following dissemination at conferences, and upon publication in peer-reviewed scientific journals, likely to take place within a year of its conclusion. In the longer term, patients may benefit from either repurposed existing therapies or from the development of new drugs which act the pathways this project will reveal. The overall aim therefore is to improve treatment for often fatal and severe disease, and to save human life.

How will you look to maximise the outputs of this work?

The results from this project will be initially communicated at scientific conferences and via publicly available pre-prints, and then in peer reviewed journals. The nature of the work is highly collaborative and will allow the building of new research teams to continue active investigation. There are peer reviewed public engagement plans in the grants supporting this project to maximise the overall sharing of the results and their impact.

Species and numbers of animals expected to be used

- Mice: 14000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are used as the lowest vertebrate with an immune system which closely approximates that of humans. The experimental protocols are well-established and validated in mice, which increases the overall reproducibility of the work. There are highly validated tools for measuring the outcomes of the project in mice. We will predominantly use adult mice with a mature immune system.

Typically, what will be done to an animal used in your project?

Mice will typically receive injections of substances used to induce an immune response, and the effect of deletion of genes involved in metabolism measured by killing the mouse and analysing its immune tissues. The span of this will typically be 2-3 weeks. Some elements of the project will use mice which develop lymphoma or autoimmune disease, and measure the impact of the disease on the metabolism of immune cells. Mice will typically be killed before they develop clinically significant disease. Other experiments will look at the effect of treatment with drugs that modulate metabolism in healthy mice and in those with lymphoma or autoimmune

disease.
What are the expected impacts and/or adverse effects for the animals during your project?

The overwhelming majority of mice will undergo either subthreshold or mild experiences, typically associated with breeding or ear clipping for genotyping or identification, and which are highly transient.

For mice on moderate protocols, the expected impacts may include weight loss, pain, tumours, diarrhoea, arthritis, and lymph node enlargement. The majority of these symptoms are relatively short in duration (<3 weeks), as the affected mice will be killed at the earliest timepoint possible whilst still obtaining reproducible data.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The great majority of mice will experience subthreshold severity. Otherwise, the severities are from mild (75%) to moderate (25%).
What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The immune response cannot be completely modelled *in vitro* due to the complex interactions between immune cells in a specific microenvironment. The same is true for lymphoma, in which *in vitro* growth significantly disrupts normal metabolism. In order to fully understand, on a mechanistic basis, the impact of pathway manipulation in an immune cell it is necessary to genetically disrupt the cell using conditional knockout techniques, which again is only fully informative *in vivo*. Finally, to determine if potential treatments for lymphoma or autoimmunity are effective, this must be done *in vivo*.
Which non-animal alternatives did you consider for use in this project?

We considered (and use) *in vitro* culture systems to measure basic elements of the immune response, and to study of lymphoma in culture. We have also searched existing databases of results which we can apply to our research questions, without needing to repeat the experiments in animals ourselves.
Why were they not suitable?

They do not fully recapitulate the complete immune response, and in particular metabolism in cell culture systems is very different to that in vivo, due to differences in oxygen concentrations and nutrient availability.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimate of mouse numbers is based on experimental experience, aiming to balance reduction in use with producing robust, reproducible, and meaningful results. Statistical techniques have been used to estimate the number of mice included in each experimental group, to reduce the risk of false positive or negative results.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used the NC3R's Experimental Design Assistant, and employed statistical calculation using analysis software.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

As a key element of the usage of mice is the breeding of genetically-altered animals, we will optimise breeding strategies to reduce surplus animals which would otherwise need to be culled. In situations where statistical calculation of group sizes are more difficult due to limited prior knowledge, we will employ pilot, preliminary experiments to reduce the risk of inadvertent harm to large groups. The same approach will be used when treating mice with therapies that have limited clinical experience. The experimental utility of each mouse will be optimised by maximising the number of measurements taken from each animal, and tissue will be shared when possible to reduce the need for mouse use by other groups.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The experimental methods and models we will employ are all well established as techniques which provide robust and reproducible results carefully balanced against causing unnecessary harm.

Strains of mice are used that delete genes only in specific types of immune cells, reducing the harm that might occur if all cells were deficient. When mice are used to model human disease, we will select strains that reproduce human disease but with the least severe symptoms. For example, this includes breeding mice which develop lupus, with those that have protective genes, producing an overall much milder model. We will also use chemical 'triggers' to delete genes only when required, avoiding the adverse effects that would occur if this had been present from birth. Wherever possible we analyse mice at a minimal symptomatic stage of the intervention or disease model, and throughout mice are strictly subject to humane endpoints to prevent excessive suffering.

An essential part of this work is to understand immune responses, usually by immunisation or infection. In all cases, we will use immunisation techniques with the least adverse effects, and infection with the weakest strains of microorganisms and at the lowest doses we can. The same principle applies when using drugs to alter metabolism or as potential therapies: the lowest effective dose will be used to minimise side effects.
Why can’t you use animals that are less sentient?

Mice are the least sentient animals which have an immune system similar enough to humans to be able to begin to generalise results, and in which human immune disease and lymphoma can be modelled. The availability of disease models allow us to test whether new treatment might be effective in humans, and whether side effects are likely.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Central to our refinement efforts is questioning whether the same experimental results can be achieved with reductions in doses of substances, over shorter periods of time, and with earlier clinical or nonclinical endpoints, such as the use of biomarkers or by imaging techniques.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the ARRIVE, PREPARE, and FELASA guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We closely follow the immunology literature relevant to the project, continuously looking for techniques which will allow us the refine the animal work which we conduct. We also following NC3Rs initiatives closely, both on their website and as disseminated through the university.

Home Office

NON-TECHNICAL SUMMARY

75. How nutrition and ecology impact infection and immunity

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Wood mice	adult, juvenile, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The central aim of this project is to better understand how natural ecological variation, including differences in nutrition, can impact parasite and pathogen dynamics and host immunity, in order to better understand the impact of infectious diseases in natural populations.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

To understand how parasites and pathogens impact the fitness and dynamics of their wild hosts, we need to recognise and investigate the ecological heterogeneities that are inherent in natural systems. Lab studies can, and do, generate invaluable information on how variation in nutrition, sex, age or past infections affect infection, immune responses and disease progression. However, there are many aspects of the controlled setting that don't capture the ecology of natural systems. Life in the wild is tough: individuals compete for food, mates and space; endure seasonal and spatial environmental variability; are exposed to a vast array of parasites; and also vary in their genetic composition. All of these factors will influence the likelihood of an individual being infected with a given parasite, the magnitude and efficacy of the immune response, and the clinical progression of disease. However, understanding the roles of each of these factors in isolation, and certainly in combination, is not logistically possible with lab studies alone; as such, we have a poor understanding of how these responses are affected by the simultaneous variation of these various factors, as happens in natural settings. If we are to understand disease progression, immunity and parasite transmission, we need to complement lab approaches with detailed studies of host-parasite interactions in their natural setting. This combined approach is essential to understand both the mechanisms driving parasite infection and immunity, and their implications for host health and population, or community, dynamics.

In our research, we complement our understanding of infection and immunity, as obtained from studies on lab mice, with experiments from wild rodent systems to provide a comprehensive understanding of what ecological factors determine infection and immunity.

What outputs do you think you will see at the end of this project?

This project will (a) advance our understanding of how the immune system operates in the wild in response to infection and coinfection, (b) determine what factors, with a specific focus on nutrition, drive individual variation in infection, disease, and treatment success, (c) strengthen the translation of immunology research to the development of medical interventions, especially in developing treatment strategies for variable host populations, and (d) better understand the patterns of cross-species transmission for multi-host parasites. By integrating field experiments with lab experiments using the same, naturally occurring, host-parasite combinations, our methods and results will have widespread benefit for health studies in non-laboratory settings, and for non-model species. Also by using genetically altered mouse strains, with known changes in their immunological phenotypes and expression, we aim to understand the mechanisms driving these important host-parasite interactions.

Thus the direct benefits of this project will be to:

- Improve current knowledge of how the immune system operates in wild populations and in controlled studies that better mimic natural ecological variation (e.g. different food quality, coinfection, etc)

- Understand how the immune system prioritises its response to different pathogens in wild, lab and GA mice;

- Understand the unintended consequences of disease intervention strategies (e.g. drug treatments, vaccination) for both host health and the dynamics of coinfecting parasites

- Better understand how different pathogens interact when body condition (sex, age, nutrition, etc.) varies across individuals and what the causes of these interactions are on host health;

- Detail how nutritional quality, availability and specific micro- and macro-nutrient quantities impact infection, immunity and response to disease control measures;

- Help identify priorities for medical intervention – for example, the proportion of individuals that should receive treatment/vaccination or how spatial movements can affect spread of disease.

- Help to test new technologies for measuring animal movement and behaviour in a UK habitat and on small rodents.

Who or what will benefit from these outputs, and how?

This project will be of most relevance to researchers working on infectious disease ecology; those interested in the spread and impact of parasites and pathogens within host populations (human, agricultural or wildlife). From an applied perspective we also see great interest to those involved in the design and outcomes of population-wide disease treatment programmes, e.g., mass drug administration, vaccination programmes, nutritional integration with mass drug administration programmes. Given that there is considerable debate on the wider benefits of both deworming and integrating deworming with nutritional supplements, and the constraints of assessing alternative treatment scenarios in human communities, we see experiments on natural mammalian host populations to be informative about the potential wider impacts of alternative treatment strategies. Furthermore, by taking wood mouse immunology into the natural setting, we envisage our work will be of great interest to immunologists in providing an opportunity to test many ideas of immune activation and action in the face of genuine parasitological and physiological challenges.

How will you look to maximise the outputs of this work?

Our regular contact with the academic research community greatly facilitate the rapid dissemination of our key findings to those working in similar areas. Beyond just publishing our results in interdisciplinary journals and giving talks we also aim to collaborate with different groups and share samples. Over the last 6 years we have provided wild rodent samples and data to >20 separate research groups and agencies (e.g., faecal samples to measure the wild mouse microbiome, blood samples to identify potential novel infections, host tissue to characterise immune diversity at key immune genes, parasite material to examine genetic diversity and for phylogenetic analyses, serum to find mRNAs as biomarkers of infection, components of the digestive tract to investigate microbiome–immune system interactions, oral swabs to assess oral disease and bone loss, ectoparasite samples to measure the incidence of Lyme disease in Scotland, etc.), which has led to a number of publications and aided the development of grant applications by several other groups. Our reputation as willing collaborators in the provision of material has grown rapidly, and we will continue to provide samples and data, benefitting the international research community and providing clear added value to our research.
We always disseminate our data and knowledge using traditional routes such as publishing the results of both

successful and unsuccessful work. We also strive to take part in many outreach activities with the public to engage adults and children in our research about infectious diseases. **Species and numbers of animals expected to be used**

- Mice: 1750
- Other rodents: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

For our laboratory work we use inbred laboratory mice as they provide a range of reagents not available in other species. In addition, we also use a laboratory colony of wild-derived wood mice, as they are the primary natural hosts for several important parasites, and as such are more appropriate models for investigating the ecology of infection and immunity. Our laboratory research using mice involves both sexes of mice, and we focus our work on adults under 6 months of age to mitigate any risk of adverse effects with helminth infections.

Wild mice and voles are natural hosts of a number of medically-relevant parasites. These species, and their parasites, have been studied for decades in the UK, and this knowledge base makes them excellent species to address our aims. Our live-trapping protocols are standard, and have been optimized to be as minimally invasive as possible. (e.g. with protective shelters, bedding and food provided). We cannot discriminate what life stage of animal we catch in our traps, but we only carry out procedures on mice over 12g.
Typically, what will be done to an animal used in your project?

For lab studies: animals will typically be ear clipped for identification if grouped housed, housed in the same cage with a partition or single housed. Animals will be infected with one or more parasite orally (e.g. helminth, protozoa), or by injection (e.g. bacteria) or intranasally (e.g. virus). Animals may be given one or multiple doses of each of these pathogens. Alongside the inoculation of none, one or more pathogen/parasite the animal may be given an immunisation (e.g. antigen, attenuated parasite, DNA vaccine, cells). Immunisations may be given multiple time through the study. A blood sample may be taken regularly (e.g. weekly, fortnightly, etc.) for measuring host and parasite responses (e.g. immunological metrics, to confirm pathogen load, host gene expression, etc.). Faecal samples maybe taken regularly to confirm gastrointestinal pathogen burdens. Animals will be killed via schedule 1 methods at the end of experiments. The duration of experiments will vary.
For field studies: animals will be trapped using live traps in their natural habitat. On the first capture, each animal will be given a tag (subcutaneous), an ear notch will be taken for DNA (both host and pathogens), a blood sample taken to measuring host and parasite responses (e.g. immunological metrics, to test for parasite infection/coinfection using diagnostics, host gene expression, etc.), and a faecal sample will be collected for host and parasite information (e.g. gastrointestinal parasites, hormone analysis, immunological analysis, etc). The animal will be weighed, sexed, measured, aged, visually inspected for ectoparasite infection, and then released at sight of capture. If caught again within a 28-day window the animal may be subjected to a tail bleed or if caught after the 28-day period the animal could be sampled via a venepuncture. Faecal samples and demographic data will regularly collect from each capture. In some experiments, we may administer one or multiple doses of drug therapies and/or be given a safe, non-toxic vaccine to stimulate the immune responses. All animals will be release at sight of capture, but in a subset of experiments, we will use schedule 1 methods to cull animals in the wild.

What are the expected impacts and/or adverse effects for the animals during your project?

For lab studies: There are a few mild symptoms that can occur from infection with various pathogens (including bacteria and viruses) and or parasites (helminths, protozoans, etc). However mice show no negative effects, and those that due, recover quickly (within 2-3 weeks). Immunocompetent mice >5 weeks of age do not usually exhibit any clinical signs. Supportive care will be provided and veterinary advice maybe sought if an animal is showing weight loss of 10% or ill health (e.g. loss of condition, piloerection, hunched posture, staining around mouth and eyes, pallor, reduced activity). If no improvement is seen within 72 hours it will be promptly killed via a schedule 1 method. Blood sampling these animals may cause mild and transient pain but this is expected to be momentary. For drug administration adverse effects could include possible erythema (reddening of the skin) from topical applications, if it ever develops, should be short-lived.
From oral application/gavage momentary discomfort, with time allowed for the animal to swallow a small volume.
For field studies; most of the studies we conduct require good recapture rates and/or repeat sampling, and so it is essential the animals remain in good health. The stresses placed on individuals are minimised through good training and efficient field technique; our techniques have been established for >10 years across various projects and are optimised to minimise stress and reduce suffering. Rarely
(<1% of captures) an animal will exhibit signs of trauma by the sampling procedure, typically being in an inert rather than an alert and active state. In such cases, the animal would first be given an opportunity to recover and if it did so immediately release. If within several minutes it failed to recover, it would be immediately euthanised by a schedule 1 method. Blood sampling these animals may cause mild and transient pain but this is expected to be momentary. For drug administration adverse effects could include possible erythema (reddening of the skin) from topical applications, if it ever develops, should be short-lived.
From oral application/gavage momentary discomfort, with time allowed for the animal to swallow a small volume.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Wood mice: 95-100% mild
Mice: 95-100% mild
Field voles: 95-100% mild
Bank voles: 95-100% mild

What will happen to animals at the end of this project?

- Kept alive
- Killed
- Set free

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our goal is to provide insight into what determines the consequences of parasite infection under real- world scenarios. Our results will improve our current knowledge of how the immune system operates and prioritises its response to different parasites in wild populations, give us a better understanding of how parasites interact in

variable ecological conditions and help identify priorities for medical intervention. Because we are interested in how whole organisms deal with natural infections, our work needs to be performed in animals. However, wherever possible we adjust the numbers of animals needed to reach robust conclusions while minimising animal usage, replace with experiments that do not involve animals when possible, and continually strive to improve our methods.

Which non-animal alternatives did you consider for use in this project?

Most of our research addresses phenomena that occur at the whole organ (e.g. spleen) or organism (e.g. effect of sex, age, etc) levels, and up to the population/community levels (e.g. disease transmission across host communities). Therefore it is not possible to use non-animal alternatives to understand how a host will respond, for example to infection with a pathogen or immunisation. Part of this project, however, aims to use data generated in animals systems to build mathematical models that will help predict how the immune system/population will perform from much fewer samples or in other systems.

Why were they not suitable?

We can use build and use mathematical models to extrapolate our results and provide insight into infectious disease in other systems, such as to aid in the design and outcomes of disease treatment programmes (e.g. deworming). However, while these mathematical models extrapolate some of our results to other systems, they rely on conducting controlled experiments in both laboratory and wild animal populations in order to develop and parameterise these models, and so the models are not suitable without the paired animal work.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

In both the laboratory and wild, we are careful to use as few animals as possible, while maximising the amount of information collected from each animal. These numbers are based on annual returns from previous PPL's where we conducted similar numbers and sizes of experiments in the laboratory in combination with large-scale field experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use appropriately controlled, replicated experiments as they provide far greater power at detecting an effect, and so require far fewer animals, than the equivalent observational study. Furthermore, our experiments are designed to minimise the numbers of animals used while maximising statistical power; the experimental designs follow close consultation with our collaborators, including experts in ecological statistics. In laboratory mice, inbred/GA lines are chosen to answer specific questions about the effects of variation (e.g. coinfection). Experimental sizes and treatment numbers will be statistical analysis. All studies are designed to conform to the requirements for statistical analysis using ANOVA or generalised linear models while minimising random effects and the need for mixed effects models. However, all wild rodent studies require taking multiple sources of variation into account; for every animal and every trapping occasion, we record a range of variables of both the

individual and the environment, to ensure our ability to statistically control for potential confounding variables. Through this we can minimise the number of animals needed in order to test the specific hypotheses of each experiment. Since the procedures are carried out as part of field sampling, the number of animals cannot be predetermined. Here, collaboration with quantitative ecologists and regular monitoring of the variability of recently collected samples help define an appropriate compromise between numbers of animals to provide statistical power, avoiding excess animal usage, and time/manpower resources.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The data generated in wild rodents will also provide datasets for computationally more demanding analyses that will allow us to identify meaningful biomarkers of immunity and health within the whole dataset. In turn, this will help reduce the number of animals needed to reach robust conclusions about, for example, treatment efficacy. We will use pilot studies to inform any new lines of studies and any methods that we employ, we will optimise so that they can be done using small volumes of blood, tissue, or faecal samples so that we can use one sample for multiple tests. We also share tissues, blood, and faecal samples with many other lab groups to ensure that all samples that we collect can be used for several scientific pursuits.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Most of the studies we conduct require good recapture rates and/or repeat sampling over time, and so it is essential the animals remain in good health. The stresses placed on individuals are minimised through good training and our efficient field techniques, which have been established for >10 years across various projects and are optimised to minimise stress and reduce suffering. Typically, >80% of resident wood mice and voles will be recaptured when trapping at 2-4 weekly intervals at the same location. Whenever treatments/procedures are administered, recapture rates of treated and control animals are compared to ensure the procedure causes no harm. In fact, both anti-parasitic treatment and nutritional supplementation are expected to improve health and survival. All participants on the project undergo rigorous training to ensure all techniques are carried out efficiently, to minimise handling and to maximise accuracy of data collection.
Wood mice and voles are natural hosts for a number of medically-relevant parasites, including *Heligomosomoides polygyrus*, a well-studied immunosuppressive helminth that is a model for human infections. These host species, and their diverse parasite and pathogen communities, have been studied for decades in the UK, and this knowledge base makes them excellent species to address our aims. We follow Natural England and Scottish Natural Heritage guidelines regarding the trapping of small mammals by setting traps as late as possible each day and checking them as early as possible the following morning, always within 16 hours of opening the traps. All traps are covered with semipermanent shelters (to reduce exposure to weather) and are provided with approved bedding material, grain, a source of moisture (e.g. vegetable/fruit) and, in areas where shrew captures are possible, a protein source (e.g. mealworms).
For our laboratory work we use a smaller number of inbred laboratory mice as they provide a range of reagents and GA animals not available in other species. Most mice used will be wild type, with occasional use of mice with alterations to genes suspected of affecting coinfection, nutrition, and/or immunity. *H. polygyrus* and *E. hungaryensis*, are two parasite species that we regularly study, are natural parasites of wood mice and have

been used as models of immunity and coinfection. Neither species is known to have an adverse effect on its host in the lab and so most of the animals undergoing these procedures, even with immunological manipulation, will typically not exhibit any discomfort. However, the majority of our experimental laboratory research will be focused on using our formerly wild now laboratory reared colony of wood mice.
In our research investigating treatments and vaccines against parasites (e.g. arthropods, helminths, protozoans, bacteria), we specifically choose drugs that are known to be effective with minimal dosing, ideally just a single dose. In all cases, the quantities administered follow recommended guidelines, have been carefully calculated to avoid toxicity, and the means of administration are both rapid and minimally invasive; favouring drugs that can be applied topically, second, orally and finally drugs applied by injection. As these treatments reduce parasite loads it is likely that such treatments are beneficial. However, in both the lab and field we closely monitor all treated and untreated animals, assessing body condition and behavioural responses immediately post-treatment and subsequently at each capture. If any animal shows any adverse response to treatment we cease treatment of that individual. If adverse reactions are extreme then the animal will be euthanised.

The criteria for their capture, manipulation and release are agreed upon with the local NVS. We are constantly refining and improving our methods, by assessing animal welfare following each procedure.

Why can’t you use animals that are less sentient?

Most of our research addresses phenomena that occur at the whole organ (e.g. spleen) or organism levels, and up to the population/community level. Because we are interested in how whole organisms deal with naturally-progressing infections, our work cannot be performed in animals that are less sentient or in cell cultures.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All experiments conducted on rodents in the laboratory will include continuous monitoring during experiments by taking their weight, fat scores and regular monitoring for changes in behaviour or appearance which would be indicative of ill health.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We use the PREPARE and ARRIVE guidelines to guide our experimental research.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay informed about advances in 3Rs through constant communication with BVS, animal units, attending workshops or seminars on the 3Rs and by periodically checking the NC3Rs website.

Home Office

NON-TECHNICAL SUMMARY

76. Identification of processes involved in blood formation and the development of blood cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

blood stem cells, blood development, blood cancer

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how blood is formed during embryonic development and how these processes can go awry, resulting in blood cancer.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Blood cells are frequently used in the clinic for cell replacement therapies, with demand often outstripping supply. A lot of research has therefore been invested in ways of generating and expanding blood cells in culture; however, robust protocols are still lacking. It is essential to understand how blood formation takes place in the embryo in order to be able to recreate these processes in culture.

A common feature of cancer is the reactivation of developmental processes, and understanding these processes can therefore aid the development of new cancer therapies. In addition, some of these early blood cells produced during foetal development have unique properties not found in adult blood cells. It is important to understand these differences in the context of infant blood cancer which starts during foetal development and which differs from blood cancer in adult patients. By identifying the factors that specifically drive the infant disease, new and more effective treatments can be developed that will benefit these very young patients who currently have a dismal prognosis.

What outputs do you think you will see at the end of this project?

We expect to gain new insights into how blood develops in the embryo and on the origins and development of infant leukaemia. These will include the description of novel mechanisms and the identification of new regulators in both of these areas. We are also hoping that these novel findings may lead to new approaches for generating blood stem cells in culture and, in the case of the infant leukaemia studies, may even lead to pre-clinical studies aimed at establishing whether any of these newly identified mechanisms and regulators may serve as novel therapeutic targets.

We will always aim to publish our findings in peer-reviewed journals and make the studies open access. In publishing the findings from our animal studies, we will make sure to follow established guidelines on how to report animal studies as detailed in the ARRIVE guidelines (https://arriveguidelines.org/). Any data sets will be deposited in appropriate repositories and made publicly available upon publication of the manuscript. Findings will also be shared at meetings and conferences, public engagement opportunities and through the University's news channels. Any factors that have shown potential in the generation and expansion of blood cells and any drugs that have shown activity in the cancer mouse models will be further pursued and advice sought on their commercial exploitation and progression to pre-clinical trials.

Who or what will benefit from these outputs, and how?

The immediate beneficiaries of our findings will be other researchers in the field who can make use of our data to progress their own research. Specifically, these will be researchers also interested in the generation and expansion of blood stem cells and those who are trying to generate blood stem cells from pluripotent stem cells in culture, which has immense clinical potential for the treatment of blood disorders. Our research may highlight new ways for them to modify their protocols, e.g. inclusion of any reagents, that could help to improve efficiency of these processes. Similarly, our studies on the origin and development of infant leukaemia, will help to drive

research forward in this field and help other researchers in putting their own findings in context with ours to gain a better understanding of the disease and identify the most promising strategies for drug development. In the long-term, we are hoping that our findings will lead to improvements in blood generation in culture and the treatment of cancer patients.

How will you look to maximise the outputs of this work?

We will always aim to make our findings open access and to make data freely accessible in repositories for the use of others. Any newly generated reagents will also be shared upon requests. We will always aim to publish all of the findings, including negative results, to avoid unnecessary duplications and to publicise the findings as much as possible through conferences and news channels.

Species and numbers of animals expected to be used

- Mice: 39700

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice have been the chosen animal model for many decades as their blood system closely resembles that of humans. Many studies on blood development, regulation and cancer have been performed using mice, which will help us to compare our results with others and put our new findings into context. The most representative models for human blood cancer have been established in mice.
An important goal in blood research is the generation of blood stem cells in culture which has still eluded the community. To be able to recreate this process in the lab, it is essential to understand how the embryo achieves this during development. This stage is very difficult to study using human material, which is why we use mouse embryos to study this.
The gold standard assay for identifying blood stem cells is transplantation into and regeneration of the entire blood system in adult recipients, which is why we need to perform this assay. In some cases, we may perform transplantations into animals before or just after they are born in order to model the migration and maturation stages that these cells need to undergo. We will also have to perform transplantation assays in order to determine whether cells can cause blood cancer. Again, we would also like to perform these transplantations using foetal or neonatal recipients as a major question of our research is the origin and development of infant blood cancer, which originates pre-birth and where the foetal context is a known contributing factor.

Typically, what will be done to an animal used in your project?

Animals in which specific genes have been altered, will be generated and bred to be able to analyse how this affects blood production and the development of cancer. For our studies on the development of the blood system, most of these animals will be culled halfway during embryonic development and their tissues used for further experiments and analyses in the lab. They may be administered with genemodifying agents orally, in the food or water or via injection. Some of these mice will be allowed to be born to assess long-term effects on the blood system or to see whether they develop blood cancer. During that time, they may be injected with labelling agents (e.g. some that label proliferating cells), with disease-initiating factors (e.g. stimulators of the immune system) or drugs that may change the disease progression.
To determine whether a cell is a stem cell or whether a cell has the potential to cause cancer, they need to be

injected into an irradiated transplant recipient to demonstrate that they can regenerate the entire blood system or cause blood cancer, respectively. Therefore, some mice, that are serving as transplant recipients, will have their own blood system removed (usually through irradiation), injected (through the vein or the bone) with donor cells and regeneration of their blood system monitored through regular blood sampling. For normal transplantation experiments, the mice need to be monitored for at least 4 months, whereas for the cancer models it depends on how quickly the cancer develops, which can be more than a year. These animals may also be given (via the most appropriate route) disease-modifying agents or therapeutic drugs. To study stem cell development in the embryo or to model infantblood cancer, it may also be necessary to perform transplantations into newborns (via injections into the facial vein or the liver) or in utero transplantations, which would require temporary exposure of the uterus by surgery.

What are the expected impacts and/or adverse effects for the animals during your project?

It is expected that suffering will be kept at a minimum since we have clearly defined end-points and scoring systems. However, some of the animals will experience transient pain through injections and blood sampling. Whole-body irradiations also lead to a temporary weight loss, but the mice will recover their initial body weight within 2 weeks of irradiation. They will also be provided with antibiotics during that period to avoid infections. Any animals undergoing more substantial procedures such as intra-bone injections and surgery will be provided with pain killers. The greatest impact will be experienced by the cancer models, but daily monitoring, regular blood sampling and scoring of the animals according to well-defined symptoms will keep discomfort at a minimum and animals killed as soon as they reach a humane end-point.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

From experience, we can predict that the vast majority (>80%) of animals will experience discomfort classified as subthreshold. Only 14% of our animals last year experienced moderate levels of severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is necessary to use animals to achieve our aims for the following reasons:
(1) Blood stem cells can only be identified through their ability to regenerate the entire blood system on a long-term basis in irradiated recipients. We routinely perform cell culture assays first in order to test novel regulators and to determine optimal treatment doses. However, these assays cannot detect true stem cells. The results from these assays normally give a good indication as to the relevance of the tested substances, but the effect on true blood stem cells will ultimately have to be established through transplantation assays.

(2) It is currently not possible to maintain blood stem cells in the lab. One of the aims of this project is the definition of the environment in which blood stem cells reside and develop. This is a complex microenvironment of cells and molecules that control blood stem cell behaviour and that cannot currently be recreated in the lab;

however, one of our goals is to define the minimal components required to maintain and expand blood stem cells in culture, which would reduce animal numbers in the future.

(3) We have recently uncovered that the development of the blood system in the embryos is influenced by the co-developing sympathetic nervous system, thus demonstrating that these developmental processes need to be studied in the context of the intact individual to reveal complex interactions between neighbouring tissues.

(4) Blood cancers are complex diseases that affect numerous tissues and organs in an individual. Disease development and progression and possible treatment strategies therefore need to be studied in an animal model; however, the initial ability of a gene to change cells in a way that might eventually lead to cancer or of a compound to affect cancer cells will be tested through experiments that can be performed in the lab rather than in animals.

Which non-animal alternatives did you consider for use in this project?

We routinely consider cell culture assays as alternatives to transplantation or to model blood stem cell formation and how this may be influenced by external signals.
We also use blood cancer cell lines derived from patients that can be grown in the lab and cell culture assays to model cell changes that could lead to cancer.

Why were they not suitable?

While cell and tissue cultures can provide a useful initial indication as to the expected effect and can be a useful platform for dose determination, effects on true blood stem cells can only be determined in transplantation assays. In addition, while some signals from the stem cell environment can be simulated in cell culture, it is currently impossible to recreate the complex environment of an organism.
Similarly, while proliferation assays in culture can give an indication if a cell is becoming cancerous and can also test whether this can be prevented through drug treatment, the full cancer and its effects on the entire organism can only be modelled in a whole organism.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

I have based this number on previous experience and the number of projects that I estimate to undertake in the following 5 years. However, before starting a new experiment we will follow established guidelines on how to prepare animal experiments (https://norecopa.no/prepare).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

When designing the experiments we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. In addition, we consider published guidelines on how to prepare animal experiments (https://norecopa.no/prepare). The number of animals to be used is estimated based on previous experience and power calculations considering the number of experiments needed and number of mice per

experiment required to obtain the appropriate statistical power. Although statistical analysis may not be completely appropriate in the consideration of all cells behavioural phenotypes, we would like to be able to assess changes in blood cell behaviour in the range of 2-fold. Power calculations around these estimates indicate group sizes in the range of 3-5 experimental and control animals. We estimate that 2-fold changes (e.g. 50% vs 25%) with a standard deviation of 10 require a sample range of 3-5 animals to give significant results ($p<0.05$) with a power of 0.80-0.97). Allowing for failures in engraftment and three analysis regimes (1 to 2 animals/analysis), the initial starting group size is 6-8 animals. In the cases where the experimental outcome of mutant cells is unknown, it is more difficult to estimate the group size required. In the cases where the experimental outcome is the development of blood cancer, it is far harder to estimate the group size required. Cancer development may occur over a long period (12-24 months) and at a frequency that is a priori unpredictable. Publications in this field generally use group sizes in the range of 10-25 animals and based on our previous experience we would regard this as a prudent and scientifically sound starting point in cases where we have no additional information to guide our experimental design.
In order to reduce the number of breeding pairs, the mice will be kept as homozygous mice, provided that they do not have a harmful phenotype.
Wherever possible potential therapeutic agents will be pre-screened to obtain indication for the minimum dose that is likely to be effective thereby reducing the numbers of animals used in pilot studies.
Mouse lines no longer required will be kept as frozen stocks.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To maximise the information from a single animal, we will aim to take samples from multiple body sites and provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. If possible and appropriate we will try to obtain tissues from other researchers who have already developed relevant mouse models.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse has been selected for this work as it is an appropriate model for haematopoiesis studies and is the species in which reliable transgene technology is best established. Since the mouse is the most commonly used model for such studies, the results can be more easily incorporated with other groups' findings and we have a large amount of background scientific data to refer to relating to the mouse.
To generate transgenic mice, inducible constructs will be used wherever possible. The mice should not display a phenotype until candidate gene expression or deletion is induced. We will only use well established reagents and protocols to induce expression or deletion of the candidate gene.
When expressing or deleting a new candidate gene, animals will be bred and analysed as heterozygous animals first to avoid unexpected pain and suffering.
When exposing mice to irradiation for creating transplant recipients, this may be administered as a split dose to give improved recovery rates.

For protocols involving bone marrow transplantation and leukaemia a stringent scoring system which allows an immediate identification of mice with adverse effects has already been implemented in our facility. This system indicates clear endpoints and will allow us to efficiently minimise animal suffering.

Why can’t you use animals that are less sentient?

Of all experimental models available, the mouse has a blood system that most closely resembles the human system. Mice have so far generated the most representative model for human blood cancers.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

As we gain experience with our leukaemia models, we can much better predict when animals will develop the disease phenotype and that way prevent unnecessary suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will consult the online resources provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research to obtain updated information on the best practices.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Throughout the project we will monitor the development of new techniques that may allow us to refine our current techniques. This information will be obtained from the literature, the veterinary service and events taking place at the University relating to the promotion of the 3R's. We have also subscribed to the monthly newsletter published by the National Centre for the Replacement, Refinement and Reduction of Animals in Research.

Home Office

NON-TECHNICAL SUMMARY

77. Imaging biomarkers of normal and abnormal brain development and ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation, or modification of physiological conditions in man, animals, or plants

Key words neurodevelopment, neurodegeneration, ageing,

imaging, brain

Animal types	Life stages
Mice	pregnant, adult, juvenile, neonate, embryo, aged
Rats	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The main aim of this project is to characterise changes in the brain that arise during normal and abnormal development and ageing. We will do this by using a battery of clinically relevant imaging and corroborative techniques, as we aim to improve the understanding of a variety of neurological disorders ranging from autism spectrum to Alzheimer's disease, in the way that matches and helps us interpret human brain imaging. Alongside this, we plan to test several novel treatments for their ability to ameliorate or halt the progression of neurodevelopment and/or neurodegeneration-related abnormalities.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In order to develop successful treatments for human neurological disorders it is important to not only model aspects of the disorders in a controlled way, but also to have the ability to detect and visualise these in a manner that is clinically translatable.

Animal models of brain disorders are a precious resource that enables us to learn more about the pathologies of the brain and the central nervous system (CNS), and how these affect the entire organism. Recent years have seen the development of sophisticated array of genetical tools with which the CNS can be manipulated in rodents that delivers a controlled and precise method for modelling pathologies, but with less overall suffering that would have been inflicted by the more traditional lesioning methods.

In this project we aim to characterise several animal models that have elements of pathologies arising from abnormal brain development and/or abnormal ageing including neural degeneration. We are particularly interested in the neurodevelopment/ageing continuum as there is a lot of evidence from clinical studies that these are connected. For example, mutations that interfere with neurodevelopment in mother's womb through childhood can be further involved in development of neurons and their connections (synaptogenesis and axon motility) eventually resulting in neurodegenerative processes later in life. In line with this it is well known there are disorders resulting from aberrant development that affect cognitive and physiological processes, and eventually progress into profound
neurodegeneration, such as for example Down's Syndrome. Ageing itself is a risk factor for developing neurodegeneration but accumulating evidence suggests there are a lot of factors influencing how well we age, which could be therapeutically modulated to ensure lifelong health and well-being. Finally, we are interested in neurodegeneration as an "accelerated ageing" process and how various abnormalities in specific proteins & neurotransmitters influence the rest of the brain and the body.

For our investigations we will use an array of non-invasive imaging methods, supplemented when appropriate by additional, corroborative techniques. Brain imaging methods, such as MRI and PET, are particularly important translational tools because of following reasons:

- They are largely non-invasive, and therefore can be employed longitudinally and without the need to sacrifice the animals and can be used to image the whole brain (not restricted to only one area) – thus ideal to monitor disease/pathology progression as well as effects of treatments.

- Multiple types of imaging markers can easily be obtained in the same session contributing toward refinement of techniques and reduction in overall animal numbers, and

- By and large, the same methods are already used in clinical (human) research, hence imaging is translational and the results from an animal study can be used to directly inform the results from a human

study.
Hence it is important to undertake this work in order to increase our understanding of the disorders that affect the brain during development and ageing, and to use the herein described methodology to test a number of experimental treatment strategies because they may lead to improved treatments for the human disorders.

What outputs do you think you will see at the end of this project?

We aim to publish all findings in quality peer-reviewed scientific journals in order to benefit the research community. In addition, the work will be presented at scientific conferences where it may be debated with and disseminated amongst other scientists working in the field of preclinical or clinical imaging and neuroscience. Ultimately, improving and validating imaging biomarkers will be possibly implemented in clinical research (early detection and monitoring of neurological diseases and their treatments), and will also have an impact on drug discovery, helping to expedite and facilitate development of new treatments for clinical disorders.

Who or what will benefit from these outputs, and how?

Short term: scientific community.
Longer term: patients undergoing imaging as part of their disease diagnosis or management.
Additionally, wider scientific imaging community may benefit through us publishing and sharing standardised protocols to avoid duplication, or obscure ‘in house’ development. As a start we have recently established such an online available platform.

How will you look to maximise the outputs of this work?

There is a drive to publish negative results across all aspects of neuroscience. Because imaging and corroborative methods are complex, time-consuming, and expensive, it is important to publish and disseminate negative results from such projects. We will endeavour to publish and disseminate these at all opportunities. Another important output strategy for our activities is via collaboration. For the same reasons outlined above (complexity, expense) most of the projects ongoing at our facility are collaborative. Our facility was created specifically to conduct collaborative projects as we have recognised that imaging experiments are multidisciplinary and must be conducted by highly experienced, often senior staff with relevant expertise, as training an inexperienced person adequately to acquire quality imaging data is a lengthy process. To this end, we strive to share our expertise in collaborative projects with neuroscientists seeking to answer questions and test hypotheses relevant to clinical scenarios using state-of-the-art translational methods.

Species and numbers of animals expected to be used

- Mice: 1450
- Rats: 1200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In this project we will use genetically modified rats and mice, as well as normal (non-GA) animals and some animals will have brain lesion induced by a toxin. All the models herein selected were chosen to enable us to study aspects of normal and abnormal brain development and ageing, as well as neurodegeneration. We will use animal models of autism spectrum disorder and of learning disability in order to learn more about abnormal brain development; these will typically be studied while juveniles or young adults, alongside normal animal counterparts of the same age. Model of Down's syndrome will be important as an example of developmental abnormalities that propagates into neurodegeneration, as most humans with Down's syndrome eventually develop Alzheimer's disease as well. Finally, we will use models of dementia, Parkinson's as well as those with degenerating white matter in the brain, in order to study abnormalities linked to later age and to this end we will study most of those animals during adulthood and older age.

Typically, what will be done to an animal used in your project?

Typically animals will be tested behaviourally to confirm the functional consequence of abnormal development or ageing/neurodegeneration. Most of the time these behavioural tests will be of mild severity; occasionally we may probe behavioural signs further by using slightly more invasive tests that involve food deprivation, exposure to water or elevated platforms. We will undertake brain imaging in the majority of animals in this project: this will be done under anaesthesia using either MRI or PET scanners. Some animals may have their brain activity recorded by inserting EEG electrodes into the brain (implanted under anaesthesia). Approximately half of animals in this project will be given substances such as contrast agents for imaging or experimental treatment drugs. These may be administered orally or by injections or by osmotic pumps inserted under the skin.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of animals will be anaesthetised repeatedly for imaging. Repeated imaging sessions are not likely to impact adversely on animal welfare. Some animals will have electrodes inserted into their brains under surgical anaesthesia, and the electrodes may be kept for several weeks. In our experience these, when implanted properly, seldom pose welfare concerns. Any animals that show any unexpected or unacceptable adverse effects will be humanely killed.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most protocols in this license are of moderate severity: this is because anaesthesia is classified as moderate. As almost all animals in this project will be anaesthetised at least once, they will undergo moderate severity procedures. Certain behavioural tests require exposure to water, or transient food restrictions which are also classified as moderate. Finally, implantation of brain electrodes and probes is also moderate. Approximately 50% of all animals in this project will undergo one anaesthesia, and approximately 50% will undergo one or more anaesthesias or one or more procedures with "moderate" severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

For our work on animal models of disorders, the animals have to be used because it is not yet possible to model the complex central nervous system (CNS) disorders in question in vitro or in silico. This is because our knowledge about structure and function of the CNS, as well as of the pathological events in these disorders is not yet sufficiently advanced. Indeed, one of the main aims of this work is to help advance knowledge about CNS disorder pathways, which will eventually lead to improved non-animal modelling of diseases.
Secondly, as this project is largely concerned with the results of disturbing the normal function of the central nervous system of living organisms, little replacement is possible. For example, we aim to investigate effects of experimental compounds on parameters such as alterations in cerebral blood flow resulting from changes in neural activity and this cannot be replicated in vitro due to a lack of adequate models of cerebral circulation. Here we aim not only to quantify parameters such as cerebral blood flow, neural activity and metabolism in areas acutely affected by the disturbance, but also their “downstream” effects (e.g. in other neural pathways) and for this there are currently no appropriate in vitro/ ex vivo replacements. However, data generated here will help to directly corroborate similar results from neuroimaging in humans (e.g. fMRI), which are still not very well understood, particularly in applied pharmacological research.

It is hoped that as more research is conducted into the uses and benefits of neuroimaging in drug development, the utilisation of the technology to safely and effectively test novel compounds in humans may become a reality. It is of course important that any experimental and novel compounds first be characterised and tested in in vitro systems and/or computer libraries. While our own laboratory is not set up to perform such assays, we only work with the compounds for which these data already exist for example we use publicly-known (characterised) compounds or obtain information from collaborators about the compounds’ previous testing and results.

Which non-animal alternatives did you consider for use in this project?

The only possible non-animal alternatives in this project are human beings.

Why were they not suitable?

This is because it is not possible, safe or ethical to conduct experiments to fulfil our aims in human beings. For example, we seek to monitor progression of disease markers, from the earliest to the later stages. This is not possible to conduct in human beings as we do not have ability to detect most brain disorders at their early stage. We are also unable due to lack of safety and ethical permission to test all experimental therapy in human beings.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

We have carefully estimated the number of animals required based on our prior experience using similar methodology and similar models. We also estimated the number of animals needed based on usage in our prior work, under previous licence.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The use of in vivo neuroimaging techniques such as fMRI and EEG in drug development and disease characterisation experiments has the potential to reduce the number of animals used due to the continuous (longitudinal) monitoring, non-invasive nature, and ability to simultaneously acquire several different measurements.

We frequently measure multiple parameters in each animal. These may include behavioural assessment, physiological parameters, cerebral metabolism, protein synthesis, blood flow, metabolites, lesion volume, size of the brain and CNS structures. Whenever possible we acquire these measurements longitudinally whereby each animal serves as its own control and through this 'within subject' design we improve statistical power. In addition, a good experimental design frequently enables us to use one control group for comparison with several intervention groups. Altogether these interventions maximise the robustness of data and sensitivity to detect significant effects, while at the same time reducing animal numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use parametric and non-parametric tests for statistical analyses, as appropriate. We base our experimental design and analyses on our long-standing experience in these research methods.
Whenever necessary, we also consult statisticians and image analysts for advice regarding design prior to commencement of novel studies. Power calculations are always conducted prior to embarking on a new study, except when attempting to detect a novel biomarker for which there are no in-house pilot data available; in this case we conduct extensive literature searches and aim to achieve comparable group size to published reports of similar studies.
Additionally, one of the aims of the current project is to collect imaging data into a database of rodent brain images at differential stages and this will be publicly shared to enable other scientists to share these data digitally.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will only use rats and mice in this project; including genetically modified. We propose these are more humane, cause far less suffering and are more precise than the more traditional models induced by lesioning. Only a small fraction of mice and rats in this project will be lesioned by a toxin, using well known established protocols, to create a model of disorders such as Parkinson's disease for which there is no suitable genetic method at the moment.
The neuroanatomy, neurophysiology, and genetics of the rat and the mouse are well understood and validated. Our laboratory has extensive experience in the use of these animals for neuroimaging and behavioural experiments. The methodology we intend to use has mostly already been optimised and validated; although we aim to occasionally develop and validate novel, and improved techniques. By doing so in commonly used rodents, these can be easily shared between the scientific community, therefore contributing to the wider refinements.
Despite the use of multiple measurements, we will not cause unnecessary suffering to the animals. Majority of our imaging methods are performed under anaesthesia; thus it is possible to acquire multiple measures without causing additional discomfort.

Only those tests that are directly appropriate to the abnormality or the circuit in question will be undertaken, and only minimum number of sessions required to produce sufficient data will be conducted, so that each animal experiences minimum required testing.
Behavioural assessments will be undertaken in order to correlate this with the brain abnormality being studied. This also increases the statistical power and improves the characterisation (multimodality) and therefore validity of a given biomarker. These behavioural paradigms are all well established.
Treatment compounds will only be tested using relevant methodology, at appropriate doses and routes of administration, and only when it is predicted that the compound might be efficacious.
All instruments are routinely calibrated and quality controlled. Data are acquired and compared to positive and negative control values, as well as assessed against known values from literature and previous experience.

Why can't you use animals that are less sentient?

For our work in animal models of brain abnormalities, the animals have to be used because it is not yet possible to model the complex CNS disorders in question in vitro or in silico. This is because our knowledge about structure and function of the CNS, as well as of the pathological events in these disorders is not yet sufficiently advanced. Indeed, one of the main aims of this work is to help advance knowledge about CNS disorder pathways, which will eventually lead to improved non-animal modelling of diseases.
In terms of sentience, rodents are the lowest stage that can be used to model fully functioning brain, in order to translate the results to humans. Many of the disorders and age-related consequences are diagnosed based on behaviour, and rodents behave is similarly enough while lower species such as fish are too phylogenetically divergent – especially for brain cortex-controlled behaviours.
Because it is imperative that we connect our results to behavioural signs, as well as to measure effects of therapy, or disease progression over time (or the combination of progression and therapy) it is not sufficient to perform imaging or recordings only once in terminally anaesthetised animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We routinely use monitoring and welfare scoring of experimental animals, analgesia, appropriate management of multiple anaesthetic sessions (e.g. administration of saline to prevent dehydration), additional environmental enrichment in cages. Post-operative care is achieved by appropriate pain management, soft bedding, liquid or soft easy-to reach food.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

NC3R and https://researchanimaltraining.com; also LASA guidance on dose selection

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Via the NC3R website and NTCO, as well as any locally or nationally organised courses. NC3R website provides information and multiple training resources as well as a newsletter. In addition I also have a beta access to the new website https://researchanimaltraining.com which provides a wealth of information and training videos.

Home Office

NON-TECHNICAL SUMMARY

78. Imaging markers of brain structure, function and pharmacology

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

imaging, brain, functional circuits, drugs, behaviour

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The main aim of the project is to characterise how the structure and function of the normal brain are affected by compounds acting on various neurotransmitters and their receptors. We aim to use clinically translatable imaging and corroborative methods to aid the interpretation of human imaging data of relevance to drug discovery in psychiatry and neurology. The project additionally aims to characterise the effects of direct and specific (opto- or chemo-genetic) manipulations of neural circuits and neurotransmitter pathways upon brain activity, to shed further light on the function of the brain and the CNS as an integrated network.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Most agents used to treat disorders of the central nervous system, as well as the disorders themselves, alter normal brain activity via direct or indirect interactions with neurotransmitter receptors or their cellular transporters. These interactions manifest as either immediate (acute) or prolonged (chronic) alterations of not only brain function, but also of brain structure (e.g. microstructural tissue changes from rearrangements of neurons, glia, or vasculature), and these effects can be visualised by state-of-the-art neuroimaging methods such as MRI and PET.
Characterising how drugs affect the brain is important for several reasons: prolonged alterations may be detrimental and present cause of concern for a particular clinical treatment (e.g. prolonged use of antipsychotics has been shown to cause microstructural brain changes), the knowledge about where and how a drug acts helps us to better understand its mode of action and the proposed clinical efficacy. Characterising downstream effects of a drug acting on a particular neurotransmitter system increases our understanding of that system, and comparing the profile of action of novel compounds to the well known “gold standard” compounds enables us to ascertain their relative pharmacological similarities. All these help to improve drug discovery programs aiming to develop new and better treatments for various human neurological and psychiatric disorders.
Because this project is concerned only with compounds that are already reasonably well characterised, and that are either already used in the clinic or are close to being clinically tested, we need to test them in living organisms as this final stage of research and development. To this end, experimental animals, and rodents in particular, represent the most appropriate systems for such testing; their brains are very well characterised and the array of methods we intend to use (imaging, behaviour, neural activity recordings, autoradiography) are widely available, standardised and translatable to the clinic.
To fulfil the aims of this project we will mostly use an array of non-invasive imaging methods, supplemented when appropriate by additional, corroborative techniques. Brain imaging methods, such as MRI and PET, are particularly important translational tools because of following reasons:

- They are largely non-invasive, and therefore can be employed longitudinally and without the need to sacrifice the animals, and can be used to image the whole brain (not restricted to only one area).
- Multiple types of imaging markers can easily be obtained in the same session contributing toward refinement of techniques and reduction in overall animal numbers.
- By and large, the same methods are already used in clinical (human) research, hence imaging is

translational and the results from an animal study can be used to directly inform the results from a human study.

The primary advantage of testing and characterising the effects of compounds, and manipulation of neural circuits in animals is that additional information can be obtained that is currently not possible to collect from human beings. To this end, we aim not only to collect an array of imaging markers from characterising the drugs in animals, but also to combine and correlate those with the more invasive in vivo measurements (EEG, microdialysis, behaviour) and with post-mortem examinations in order to gain better understanding of the cellular and molecular correlates of imaging results.

What outputs do you think you will see at the end of this project?

We aim to publish all findings in quality peer-reviewed scientific articles in order to benefit the research community. In addition, the work will be presented at scientific conferences where it may be debated with and disseminated amongst other scientists working in the field of preclinical or clinical imaging and neuroscience. Ultimately, improving and validating imaging biomarkers will be possibly implemented in clinical research and should have an impact on drug discovery, helping to expedite and facilitate development of new treatments for clinical disorders.

Who or what will benefit from these outputs, and how?

Short term: scientific community and pharmaceutical industry (drug discovery).
Longer term: patients undergoing imaging as part of their disease diagnosis or management.
Additionally, wider scientific imaging community may benefit through us publishing and sharing standardised protocols to avoid duplication, or obscure ‘in house’ development, as a start we have recently established such an online available platform.

How will you look to maximise the outputs of this work?

There is a drive to publish negative results across all aspects of neuroscience. Because imaging and corroborative methods are complex, time-consuming and expensive, it is important to publish and disseminate negative results from such projects. We will endeavour to publish and disseminate these at all opportunities.

Another important output strategy for our activities is via collaboration. For the same reasons outlined above (complexity, expense) most of the projects ongoing at our facility are collaborative. Our facility was created specifically to conduct collaborative projects as we have recognised that imaging experiments are multidisciplinary and must be conducted by highly experienced, often senior staff with relevant expertise, as training an inexperienced person adequately to acquire quality imaging data is a lengthy process. To this end, we strive to share our expertise in collaborative projects with neuroscientists seeking to answer questions and test hypotheses relevant to clinical scenarios using state-of-the-art translational methods.

Species and numbers of animals expected to be used

- Mice: 500
- Rats: 900

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will only use normal adult rodents (rats and mice). This is because we are primarily concerned with studying the normal brain and how drugs, e.g. therapeutic or novel compounds, interact with the function and structure of the brain. Rodents are suitable for this project because their brains are already very well characterised and a lot is already known about the relevant circuits and pathways. To this end, normal adult rodents represent the best system for us to test these compounds in the final stages before they are potentially used in human beings.

Typically, what will be done to an animal used in your project?

Typically, the animals will be administered with a drug of interest (by injections or by food or water) and their brains will be scanned by MRI or PET which will be done under anaesthesia. We may also perform some behavioural testing to see how the relevant drugs affect their cognition, mood, general and fine movement. Occasionally, in order to delve deeper in the brain and neural circuits, we may study the animals conscious by measuring their brain activity via EEG electrodes, or by injecting them with radioactive probes, and sacrificing, then examining the brain in detail.

What are the expected impacts and/or adverse effects for the animals during your project?

Most drugs we will study will not cause any adverse effects. Occasionally, they may make animals mildly lethargic, drowsy or hyperactive. This is not expected to cause any lasting suffering. Repeated imaging sessions will not impact adversely on animal welfare except that they will be repeatedly anaesthetised. Some animals will have electrodes inserted into their brains under surgical anaesthesia, and the electrodes may be kept for several weeks. In our experience these, when implanted properly, seldom pose welfare concerns. Any animals that show any unexpected or unacceptable adverse effects will be humanely killed.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All protocols in this license are of moderate severity: this is because anaesthesia is classified as moderate. As almost all animals in this project will be anaesthetised at least once, they will all undergo moderate severity procedures. Certain behavioural tests require exposure to water, or transient food restrictions which are also classified as moderate. Finally, implantation of brain electrodes and probes is also moderate. Approximately 50% of all animals in this project will undergo one anaesthesia, and approximately 50% will undergo one or more anaesthesias or one or more procedures with "moderate" severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

For our work on imaging the normal brain and characterisation of the compounds, animals have to be used because it is not yet possible to model the complexity of the CNS in vitro or in silico. This is because our

knowledge about structure and function of the CNS is not yet sufficiently advanced. It is important to note that all compounds we intend to use in this project have already been tested and characterised using various computer-based and in vitro methods. This particular stage of preclinical in vivo imaging and behaviour/brain activity recording typically represents the final phase before such experiments are performed in human beings.
As this project is largely concerned with characterising the changes in normal function of the central nervous system of living organisms, little replacement is possible. For example, we aim to investigate effects of experimental compounds on parameters such as alterations in cerebral blood flow resulting from changes in neural activity and this cannot be replicated in vitro due to a lack of adequate models of cerebral circulation. Here we aim not only to quantify parameters such as cerebral blood flow, neural activity and metabolism in areas acutely affected by the disturbance, but also their "downstream" effects
(e.g. in other neural pathways) and for this there are currently no appropriate in vitro/ex vivo replacements. However, data generated here will help to directly corroborate similar results from neuroimaging in humans (e.g. fMRI), which are still not very well understood, particularly in applied pharmacological research.

It is hoped that as more research is conducted into the uses and benefits of neuroimaging in drug development, the utilisation of the technology to safely and effectively test novel compounds in humans may become a reality. It is of course important that any experimental and novel compounds first be characterised and tested in in vitro systems and/or computer libraries. While our own laboratory is not set up to perform such assays, we only work with the compounds for which these data already exist for example we use publicly-known (characterised) compounds or obtain information from collaborators about the compounds' previous testing and results.

Which non-animal alternatives did you consider for use in this project?

It is not possible to use non-animal alternatives for this project.

Why were they not suitable?

This is because there are no model systems that can adequately model the living brain for imaging biomarker development. Human beings are being used to some extent, but we cannot perform invasive investigations or take brain samples preventing us from being able to determine underlying biological correlates of imaging results.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have carefully estimated the number of animals required based on prior experience using similar methodology. We also estimated the number of animals needed based on usage in our prior work, under previous PPL.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The use of in vivo neuroimaging techniques such as fMRI and EEG in drug development and brain

function/structure characterisation experiments has the potential to reduce the number of animals used due to the continuous (longitudinal) monitoring, non-invasive nature, and ability to simultaneously acquire several different measurements.
We frequently measure multiple parameters in each animal. These may include behavioural assessment, physiological parameters, cerebral metabolism, protein synthesis, blood flow, metabolites, size of the brain and CNS structures. Whenever appropriate we acquire these measurements longitudinally whereby each animal serves as its own control and through this 'within subject' design we improve statistical power. In addition, a good experimental design frequently enables us to use one control group for comparison with several intervention groups. Altogether these interventions maximise the robustness of data and sensitivity to detect significant effects, while at the same time reducing animal numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use parametric and non-parametric tests for statistical analyses, as appropriate. We base our experimental design and analyses on our long-standing experience in these research methods.
Whenever necessary, we also consult statisticians and image analysts for advice regarding design prior to commencement of novel studies. Power calculations are always conducted prior to embarking on a new study, except when attempting to detect a novel biomarker for which there are no in-house pilot data available; in this case we conduct extensive literature searches and aim to achieve comparable group size to published reports of similar studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will only use normal adult rats and mice in this project.
The neuroanatomy, neurophysiology, and genetics of the rat and the mouse are well understood and validated. Our laboratory has extensive experience in the use of these animals for neuroimaging and behavioural experiments. The methodology we intend to use has mostly already been optimised and validated; although we aim to occasionally develop and validate novel, and improved techniques. By doing so in commonly used rodents, these can be easily shared between the scientific community, therefore contributing to the wider refinements.
Despite the use of multiple measurements, we will not cause unnecessary suffering to the animals. Majority of our imaging methods are performed under anaesthesia; thus it is possible to acquire multiple measures without causing additional discomfort.
Only those tests that are directly appropriate to the compound or the circuit in question will be undertaken, and only minimum number of sessions required to produce sufficient data will be conducted, so that each animal experiences minimum required testing.
Behavioural assessments will be undertaken when there is unsatisfactory existing data characterising a given compound or intervention, or when we need to correlate it with imaging or neural recording data, thus increasing the statistical power or improving the characterisation (multimodality) and therefore validity of a given biomarker. These behavioural paradigms are all well established.
Compounds will only be tested using relevant methodology, at appropriate doses and routes of administration, and only when it is predicted that the compound might be efficacious.

All instruments are routinely calibrated and quality controlled. Data are acquired and compared to positive and negative control values, as well as assessed against known values from literature and previous experience.

Why can’t you use animals that are less sentient?

For our work on normal and altered brain structure and function, and how the changes relate to behaviour, the animals have to be used because it is not yet possible to model the complexity of CNS in vitro or in silico. This is because our knowledge about structure and function of the CNS is not yet sufficiently advanced.
In terms of sentience, rodents are the lowest stage that can be used to model a fully functioning brain, in order to translate the results to humans. Because it is often important that we connect our results to behavioural signs, as well as to measure effects of therapy, or intervention over time it is not always sufficient to perform imaging or recordings only once in terminally anaesthetised animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We routinely use monitoring and welfare scoring of experimental animals, analgesia, appropriate management of multiple anaesthetic sessions (e.g. administration of saline to prevent dehydration), additional environmental enrichment in cages. Post operative care is achieved by appropriate pain management, soft bedding, liquid or soft easy-to reach food.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

NC3R and https://researchanimaltraining.com; also LASA guidance on dose selection.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Via the NC3R website and NTCO, as well as any locally or nationally organised courses. NC3R website provides information and multiple training resources as well as a newsletter. In addition I also have a beta access to the new website https://researchanimaltraining.com which provides a wealth of information and training videos.

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NON-TECHNICAL SUMMARY

79. Immune cell migration in health, age and inflammatory arthritis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

inflammation, leukocyte trafficking, ageing, arthritis, therapy

Animal types	Life stages
Mice	juvenile, adult, pregnant, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project explores the processes controlling the movement of immune cells from the blood into tissue in health, how these goes wrong in age and inflammatory arthritis; and it also tests the ability of new chemical and cell-based therapies to restore normal immune cell movement.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Immune cell movement from the blood into tissues is an important protective response to infection and injury. It is very tightly controlled, like security checkpoints, to prevent unwanted inflammatory responses. However, many of these security checkpoints are lost in chronic diseases such as rheumatoid arthritis and cancer, but also as we age. Our work aims to provide more detailed knowledge on the factors responsible for maintaining these security checkpoints; exactly how the security checkpoints are altered during the ageing process and in chronic disease, and if certain new chemical or cell-based therapies can restore the normal function of these checkpoints. Ultimately our aim is to use this knowledge to develop a new type of treatment that treats the cause of abnormal immune cell movement, rather than treating the symptoms that arise from unwanted inflammatory responses.

What outputs do you think you will see at the end of this project?

Project outputs will include:

- advances in scientific knowledge on the processes controlling immune cell movement in health, with age and in disease; scientific and lay publications of our findings and methodology;
- presentation of our data and/or methodology to the wider scientific community through conference oral and poster presentations;
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- identification of novel targets or agents that alter immune cell movement, which can be take forward to develop new therapies.

Who or what will benefit from these outputs, and how?

This project has a wide range of academic beneficiaries.

Short-medium term

Scientists and innovations in analysing immune cell movement in health, ageing and chronic disease described in this licence will have an immediate impact on the ongoing projects at the host organisation, and also further afield upon dissemination. Moreover, this project aims to improve our understanding the processes driving pathology in ageing and chronic disease, specifically linked to the loss of an endogenous regulatory pathway, an area of intense research interest worldwide. The impact of this is likely to occur in the short-to medium term, during the lifetime of this project and beyond as all publications are released.

Medium-long term

Collaborations – We intend to invite external seminar speakers who have an interest in immune cell movement, ageing and chronic inflammatory diseases to our organisation, with the view of fostering further collaborations based on the concepts and ideas incorporated in this proposal. We envisage that these collaborations will occur

during and following the completion of this project, and therefore represent a medium to long-term impact of this work.

Long term

Clinical Academics and pharmaceutical companies will benefit from advances in our understanding of disease pathology and premature ageing, along with our identification of new targets for drug development. We are in continual conversations with these collaborators and view the translational of our findings into new drugs over the subsequent 10-15 years following the completion of this project representing the long-term impact of this project.

How will you look to maximise the outputs of this work?

Dissemination of information

We will work with the relevant teams at our Institute to facilitate communications and resulting impact. We plan to use several routes to disseminate our findings to the wider scientific community, industry and the public that will facilitate end-user engagement:

(a) Peer-reviewed publication. We aim to publish high impact papers based on the findings generated from the research grants funding this project licence. In addition, our group has a strong tradition of publishing methodology papers; and negative data to ensure that groups do not unnecessarily repeat experiments that either technically are flawed or biologically yield the null hypothesis.

(b) Presentations. We and our collaborators will present data at internal seminars along with national and international conferences, such as British Society of Immunology (BSI); BSR; Society of Leukocyte Biology (SLB); Society of Cell Biology Research.

(c) Dissemination via international societies. We and our collaborators are active members of various scientific societies, such as BSI; BSR; SLB; European Workshop for Rheumatology Research; British Society for Ageing Research, allowing our findings to be disseminated to the wider scientific community in societal magazines and training workshops.

Enhancement of public understanding and engagement with research

We will take advantage of several events organised by the Public Engagement Working Group at our organisation and local charities to facilitate the public's awareness our research:

(a) Science "pop-up" activities. We will give at least three research talks at student recruitment days and will be participate in "Meet the Scientist" tours. We will aim to host at least 1 stall at the Annual Community Awareness Day and host an event local Science Festivals.

(b) Lay Resources. We would develop lay resources, in collaboration with our patient/public research partners (PRP), for publication of Atlas of Science. Additionally, we will continue to involve patients and the public in the delivery and dissemination of research generated from this project.

Clinical Collaboration.

My team and I are active members of several multi-institute research centres and will be able to present our findings at least twice annually at ongoing Centre seminars. We will also attend clinical conferences [e.g. European League Against Rheumatism (EULAR), American College of Rheumatology (ACR), British Society of Rheumatology (BSR)], where we will present our data and foster

collaborative opportunities for translational research across the fields of rheumatology, ageing and chronic inflammatory diseases.

Species and numbers of animals expected to be used

- Mice: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We have chosen the types of animals and their age based on our need to model a mammalian musculoskeletal aging and disease that occurs in adult humans. As such, adult mice represent the lowest mammalian option available with a musculoskeletal system in which we can study immune cell movement in health, with age and in disease, and to test potential drugs for human use.

Typically, what will be done to an animal used in your project?

Animals will have either have arthritis induced (~70%) or ovaries removed (~30%). Test agents that might limit the movement of immune cells into tissues (e.g. the joints) will be given, and the tissues monitored during the inflammatory response.

For arthritis models, the majority of animals (80%) will undergo general anaesthesia (up to 30 minutes), daily handling for scoring and caliper measurements of joints and limbs; administration of an agent (typically x15 injections), dynamic weight bearing analysis (optional for monoarthritis) and will be killed up to week 8 via Schedule 1 method or non-schedule 1 for withdrawal of fluids (e.g. blood).

Alternatively, animals (100%) will undergo general anaesthesia (up to 60 minutes), ovariectomy surgery; administration of an agent (typically x15 injections) and will be killed up to week 6 via Schedule 1 method or non-schedule 1 for withdrawal of fluids (e.g. blood).

What are the expected impacts and/or adverse effects for the animals during your project?

All forms of arthritis cause joint stiffness, pain and some degree of disability. Animals may also lose weight or show signs of abnormal subdued behaviour. Arthritis, weight and behaviour will be monitored carefully using a scoring system that details when humane endpoints need to be actioned. Pain will be managed with pre-emptive pain relief.

For the age-related musculoskeletal model it is expects that animals will experience a low level of pain shortly after the surgical procedures which will be managed with pain relief. Breathing problems may occur following inhalation anaesthesia and leading to listlessness and laboured respiration. Such impacts/adverse effects will be addressed as described for up to 24 hours before humane endpoints are actioned.

We anticipate no adverse effects of the administered agents beyond those experienced during the administration step.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Moderate severity to approximately 95% of animals Mild

severity to approximately 5% of animals.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The steps leading to the development of the state of chronic inflammation are poorly understood and it is likely that multiple physiological processes are involved, including proliferation of precursor cells, activation of lymphocytes and recruitment of inflammatory cells to the site of inflammation. Age-related musculoskeletal diseases, such as rheumatoid arthritis, are not static diseases confined to a single tissue or time point and hence modelling the effects of specific regulatory processes and new therapies inevitably involves the use of whole organisms and, in particular, the use of animal models of inflammation. However, where possible, cells from patients will be used to address questions relating to the mechanisms of action of therapeutic targets (see below in vitro models).

Which non-animal alternatives did you consider for use in this project?

We have pioneered a range of in vitro multi-cellular 3D constructs, incorporating primary human cells/tissues from healthy subjects and patients with different types of inflammatory arthritis. This has allowed us to further our understanding of the mechanisms regulating leukocyte recruitment. Indeed, for the majority of our work to date, we have used these in vitro tissue culture techniques to provide information about molecular and cellular mechanism(s) involved in the pathogenesis of disease or therapeutic effects. These cell culture assays will continued to be used throughout this licence to assess potential therapeutic molecules in functional assays such as expression, activation, proliferation and cytotoxicity. Molecules shown to be inactive in these assays will not be examined further in vivo. This utilisation of in vitro assays is an important replacement of in vivo experiments. Moreover, key molecules and/or processes identified using human and patient material will be used to inform the in vivo studies, focusing on those aspects relevant to the disease setting we are modelling.

Why were they not suitable?

Our in vitro models are unable to fully recapitulate the blood and lymphatic vasculature, and the movement of cells through complex organs, Moreover, they must be validated in licensing authority approved animal models before they enter clinical trials and are tested on patients. In some cases, this work represents preclinical therapeutic (drug and/or cell) efficacy studies required prior to embarking on toxicology studies and human clinical trials. There are no other in vitro or in vivo alternatives to this work. We will continue to collect as much in vitro evidence as possible before embarking on animal experiments, using it to inform and refine experimental design.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have used specific mathematical calculations based upon previous studies and the likelihood of our interventions producing positive results, to estimate the number of animals we will use in our study. For all experimentation, the lowest possible number of animals will be tested whilst ensuring that the experimental result is robust.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used statistical analysis to calculate the minimum number of animals necessary for this project.
We will continue to use the NC3R's experimental design tool to aid experimental design and consult trained statisticians before using any new protocols.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have used statistical analysis to calculate the minimum number of animals necessary for each experiment within this project.
New interventions will first be tested for efficacy using in vitro models prior to use in vivo. Where new routes of administration or new interventions are being examined, pilot studies will first be established in 2-3 mice prior to full experiments. Subsequently these pilot data will used in the specific mathematical calculations described above to ensure that we use the minimum number of animals needed to obtain statistically significant results.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice will be used in arthritis models affecting either one joint, or multiple joints, and also in an age-related musculoskeletal model associated with age-related changes in immune cell movement. The single joint arthritic model offers the opportunity to significantly reduce the suffering and harm to the animal induced by inflammation in many joints. This is the preferred model of choice unless the model fails to recapitulate what we have already observed using the multiple joint arthritic model. This species and procedures have been chosen as they represent the lowest animal models with a musculoskeletal system in which it is possible to study immune cell

movement in health, with age and in disease, and the effects of a human protein have on this.

Why can’t you use animals that are less sentient?

Less sentient animals do not possess the same sort of skeletal structure that composes the joints, and often their vascular tree and immune system do not fully represent that of humans. Small rodents are the lowest mammals that can be used to recapitulate the human immune systems response to joint inflammation.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Each experimental model will be monitored daily following intervention and mice will be assessed for any signs of distress such as pain and inability to feed. Wet food will be given for mice with diarrhoea and barrier cream will be used prophylactically to prevent skin irritation. Surgical interventions will be undertaken using the most appropriate anaesthetic and analgesia will be given. The mode of substance administration will be chosen to cause the least harm and distress to the animal. Any new substances or route of administration will be tested in a small pilot study and the mice monitored daily for signs of distress. Humane endpoints will be strictly adhered to at all times.
We have extensively refined the scoring system for each individual polyarthritis model to capture the specific aspects of each arthritis phenotype, ensuring clear and consistent analgesia and humane endpoints. We have also made refinements to the housing of the animals to cater for any disability arising from arthritis - including soft flooring, non-tangling nesting material, long spouts on water bottles, food on the cage floor.
We will also systemically review each experiment on completion to see what lessons can be learned from the study in terms of endpoints (scientific and humane) and any animal welfare issues that may have arisen during the experiment that could then guide the subsequent experiments.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Animal welfare is a key consideration in all of our protocols, and we will be guided by our NACWOs and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. Following every experiment and regularly during group meetings we will review our procedures from a welfare standpoint to identify any potential for refinement.
We will also follow the published literature on arthritic models - for example the case for using different arthritis models to model the different aspects of inflammatory pathogenesis is extremely well made and described in Vincent et al. 2012. Moreover, refinement of the CIA injections has been described in Hawkins et al. (2015) and we have implemented these in our work to date and will continue to do so in this project.
If undertaking a systematic review, we will use SyRF, the free online platform for researchers, to perform a systematic review and meta-analysis of animal studies. This will allow us to keep up to date with any improvements in protocols and techniques which may reduce or replace the use of animals.
Finally, we will follow the LASA guidelines Guiding Principles for Preparing for and Undertaking Aseptic Surgery (www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf) and by the Home Office Minimum Standards for Aseptic Surgery (www.procedureswithcare.org.uk/ASMS2012.pdf) when undertaking aseptic surgery and providing analgesia.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

We will continue to engage with Institutional efforts to promote the 3Rs and workshops; and receive the NC3Rs newsletter.

Home Office

NON-TECHNICAL SUMMARY

80. Immune mediated disease and immunomodulation by parasites

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

Helminth, Allergy, Inflammation, Obesity, IL-33

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this project is to determine how immune responses are controlled in parasitic infection and immune-mediated disease, by the host and by immunomodulatory products released by the parasite. We will identify, characterise and develop parasite immunomodulatory molecules for potential use as therapeutic agents in human allergic, inflammatory and metabolic disease, with a particular focus on the IL-33 pathway.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Helminth parasites (parasitic worms) infect more than a quarter of the world's population, and have significant economic impacts on livestock rearing. The immune system is capable of ejection and killing of helminths parasites, however these parasites have developed very sophisticated techniques for suppressing immune responses against them, allowing them to survive in the host. In this project, we will investigate how the immune system ejects parasitic worms, how parasitic worms suppress the immune response, how parasitic immuno-suppression could be used to treat allergic and inflammatory diseases, and how the anti-parasite immune response could prevent weight gain in obesity.

Human population which have high levels of parasitic infections tend to have low levels of diseases caused by a hyperactive immune response such as allergies, autoimmunities and inflammatory diseases, as well as lower levels of obesity. We believe that this is due to the parasite releasing products into the host which suppress the immune response - over the past 5 years we have identified several individual proteins secreted by the parasite and shown that these can suppress inflammation in mouse models of asthma.

We now plan to identify further products released by the parasite which reduce disease in models of asthma, as well as showing that previously- and newly-identified products also suppress disease in models of asthma, inflammation, pneumonia, and obesity.

Much of our work will focus on the IL-33 pathway. IL-33 is an important messenger molecule in the immune system, and our previous work shows that it is the target of a series of immunosuppressive products from a parasitic worm. IL-33 is released on tissue damage, and initiates immune responses. IL-33 is important in a wide range of diseases, including allergies, asthma, pneumonia and obesity (amongst many others). However, we will not limit our research to the IL-33 pathway, but also assess the effects of parasite products on other relevant immune pathways.

What outputs do you think you will see at the end of this project?

The main output from this project will be better scientific understanding of how parasitic worms control (and are controlled by) the immune system. We will also investigate the role of blocking parasite interactions with the host immune system, as a proof of principle that this could be used as a vaccination strategy - if successful, this could ultimately lead to the development of new vaccines against parasitic worms which affect millions of people, as well as livestock and companion animals. We will investigate the products that parasites secrete to control the immune system - we plan to use this knowledge to develop new treatments for human immune-mediated diseases, including asthma, allergies, acute lung injury, pneumonia and obesity.

Who or what will benefit from these outputs, and how?

Ultimately, this project could benefit the many millions of people infected with parasitic worms in the world, through the development of vaccines. It could also benefit society economically, as parasitic infections of livestock have a large economic impact on meat and dairy production. Development of new treatments for asthma could benefit the 1 in 11 people in the UK who live with asthma, while better treatments are required for acute lung injury and pneumonia, diseases with high mortality rates which kill millions of people around the world every year. The obesity epidemic in the western world continues to increase, and hundreds of millions of people around the world are now classed as obese - better understanding of the biological processes that control obesity could have enormous health and economic benefits.

How will you look to maximise the outputs of this work?

We will at all times seek the best collaborations available. We currently have collaborations with leaders in the fields of parasite immunomodulation, anti-parasite immune responses and immune responses in fatty tissue. As further avenues of research become available, we will seek out collaborations to further this work.
Our main outputs from our research will be in the form of peer-reviewed publications in scientific journals, and we will seek to publish all data, positive or negative. However we have also always, and will continue to seek public engagement opportunities and to produce press releases for general news publications.

Species and numbers of animals expected to be used

- Mice: 10000
- Rats: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will focus on how the immune system kills parasitic worms, and how parasitic worms suppress the immune system, and suppress diseases caused by the immune system such as allergic asthma. We will also assess how immune responses to parasites prevent obesity. To investigate this, we will use mouse models of parasitic infection, asthma, acute lung injury, pneumonia and obesity.
Mice are the best model of the human immune system - we understand the mouse immune system better than any other mammal, and a huge number of genetically modified mouse strains are available which allow us to remove certain parts of the immune response, to see how this affects infection, allergies and inflammation. We have already discovered a lot about the interaction between parasites and the immune system by using mouse models, and our lab has found several proteins secreted by parasites which could be developed as treatments for human diseases.
We will use young adult mice (6-14 weeks old) as the immune system is mature in mice of this age, and is highly responsive to stimuli.

Typically, what will be done to an animal used in your project?

Mice will be infected with parasitic worms: these parasites (depending on species) infect either directly by being swallowed, or by travelling through the skin, blood and lung, before maturing in the intestine. These infections can take between 1 week and 4 weeks. We will identify molecules which parasites secrete to suppress the immune response, and determine the effects of these by blocking their effects during infection.
We will also administer particular chemicals that cause specific types of inflammation into the lungs of mice to produce a response similar to that seen in asthma: these chemicals are that come from allergens from mould or dust, which result in the development of an allergic response. We will use parasitic infections, or injection of individual products which we have identified from parasites, to try to treat or prevent the asthma-like disease.
We will also introduce a material that comes from bacteria into the lungs of mice to cause pneumonia, or chemical agents to injure the lung (as is seen when people inhale stomach contents, potentially fatally injuring their lungs). Again, we will use parasites or their products to try and prevent or treat these responses.
Finally, we will provide mice with a high-fat diet to induce obesity. We will then infect these mice with parasites to see whether the immune response to the parasite has the side-effect of reducing weight gain.
In all of the systems above, we will use genetically-modified mice to remove or change the immune response, and see how this affects the interaction between parasites and their host.

What are the expected impacts and/or adverse effects for the animals during your project?

Parasitic infections generally do cause ill-health in infected mammals - most mice in the wild are constantly exposed to the parasites we will use, and around a billion people in the world are exposed to similar parasites, with comparatively low levels of illness caused. In some cases, however, especially when the immune response has been altered, we may see excess inflammation in the intestine or in other sites where the parasite has damaged. This can cause some weight loss and probably abdominal pain in these mice, however this is rare and short-lived - generally if we see these effects they are only for 2-3 days of the infection.
Models of asthma cause inflammation in the lung, however this is generally very mild and it is not possible to tell when a mouse has "asthma". The allergens used to cause asthma can be damaging to the lung, and so mice can have difficulty breathing immediately after receiving these but they don't appear distressed by this. These effects are short-lived however, and by the following day mice are always fully recovered.
Models of lung injury can also cause either immediate damage to the lung, or progressive inflammation during bacterial infections. Lung infections can eventually prove fatal to the mouse, however we will only be looking at the very earliest phase of infection, using a part of the bacteria rather than a bacteria that can multiply and spread, long before severe ill-health has been caused.
Finally, we will use a high-fat diet to cause obesity in mice. Although the mice will become significantly overweight, they should suffer no other ill-effects.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Protocol 1: None of the genetically modified mice we are breeding on this protocol have harmful phenotypes, therefore we expect 99% of mice on this protocol to have a sub-threshold severity.
Protocol 2: Parasitic infections in mice and rats have a moderate severity limit. We estimate that around 10% of mice infected will reach moderate severity, the remainder showing mild severity. We estimate that <1% of rats on this protocol will reach moderate severity, the remainder showing mild severit**y.**
Protocol 3: Models of asthma, acute lung injury and pneumonia have a moderate severity limit, and due to the

pathology caused by administration of the Alternaria (asthma causing) allergen, HCl treatment (mimicking stomach content inhalation) and bacterial pneumonia, we expect around 40% of mice on this protocol to reach moderate severity, the remainder showing mild severity.
Protocol 4: Models of obesity have a moderate severity limit. We do not expect any ill-effects due to diet-induced obesity, but in combination with parasitic infection and immunomodulatory blockade, we expect that up to 5% of mice may show moderate severity, the remainder showing mild severity.
Overall, we expect the following severity categories:
Rats: Mild 99%, moderate 1%.
Mice: Sub-threshold 90%, mild 8%, moderate 2%.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project will assess how parasitic worms suppress the host immune response, and how the immune response ejects parasitic helminths. It will also investigate the use of parasitic infection and molecules identified from helminths to treat diseases caused by an aberrant immune response including allergic asthma, acute lung injury, pneumonia and obesity. These systems all depend on a fundamental understanding of the immune system, which cannot be fully recapitulated outside of a mammalian organism. Therefore, as we break new ground in our understanding, we must use animals as a model of our own immune response.

Which non-animal alternatives did you consider for use in this project?

We will use in vitro systems wherever possible, however these systems are necessarily reductionist, only modelling specific individual pathways of the immune response. Therefore we can use these only when we have specific questions about a specific pathway. As we develop new and improved forms of the parasite immunomodulators, for development towards human therapeutic agents, these in vitro assays will be extremely useful for testing. However for testing factors such as immunogenicity and duration of action, these must again be tested in vivo.

Why were they not suitable?

As described above, when a system is incompletely understood (as is the case in the immune system) it cannot be fully modelled in reductionist in vitro assays. Therefore our hypotheses must be tested in vivo.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These numbers are estimates based on the number of experiments on planned projects and those already underway. The vast majority of mice will be from transgenic breeding, and the extent of these experiments will depend on success of current funding applications.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Power calculations were carried out to find the lowest numbers of animals that can be used to achieve statistical significance. Of note, we will always pool data from repeat experiments to achieve significance: many journals in the field of immunology will not publish data taken from single experiments, therefore carrying out 2 pooled repeats is the most efficient way to achieve significance.
When we have multiple treatments to test, we will try wherever possible to trial multiple treatments in the same experiments: this has the advantage that these can then be compared to the same control groups, again reducing mouse numbers used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding of transgenic mice will be kept to the minimum numbers possible, however due to complex breeding regimens of genetically-engineered mice, not all mice bred will be of a usable genetic makeup – these mice will be screened and mice which cannot be used for experimentation will be culled at the earliest possible timepoint. Wherever possible, we will investigate hypotheses in lab experiments that do not involve procedures in live mice. We have developed a series of assays which replicate key parts of the mouse models we use, which we can now use to screen molecules and test hypotheses in the lab, without the use of live mice. We will continue to develop these assays wherever possible to minimise our animal use.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use models of parasitic infection, asthma, acute lung injury, pneumonia and obesity in this project. These models have been selected to best replicate the immune response seen in human diseases, and thus results we obtain can be most readily translated to human treatments. Sometimes this means that models are used which cause greater distress to animals than some other available models, but which are more clinically

relevant. An example of this are models of asthma. A simple model of asthma is the OVA-alum model, where a single intraperitoneal injection of OVA protein in an alum adjuvant induces an allergic immune response which can be recalled in the lungs - this model causes minimal pain and suffering to animals. However, this is entirely non-physiological and is not how asthma occurs in humans. We instead will use models where clinically relevant allergens (moulds, dust) or chemicals that cause types of inflammation are introduced directly into the lungs, causing some damage and pain to the animal, but inducing an allergic or inflammatory immune response via the same pathways that are known to be important in human disease.
Wherever possible, we will use techniques that cause the least distress to animals as they are carried out. An example of this is administration of substances to the airways: previously, intratracheal administration (involving introducing a tube down the airway of an anaesthetised animal) was used which caused some inflammation in the airways, presumably with some discomfort for the animal. In all experiments involving airway administration, other than direct damage, we have now changed to using either intranasal or oropharyngeal administration: these techniques use very rapid inhalational anaesthesia, followed by administration of substances to the nostrils of the throat, and inhalation through normal respiration. This process takes less than 1 minute, is easy to master, and results in mice which have recovered fully within 5 minutes.
Models of acute lung injury by definition cause some injury to the lungs of mice. However we will assess responses over the earliest timepoints after injury, to minimise suffering. In some of these models, pathology is progressive and could ultimately lead to the death of mice from day 2 onwards if responses were left unchecked. In all of the experiments on this project, we will cull mice at a maximum of 48h after administration, at which point animals will experience only moderate ill-health. The majority of these experiments are planned to take place within 6h of administration, when the least suffering has been experienced.

Why can’t you use animals that are less sentient?

These experiments depend on a functional mammalian immune system - there are no less sentient model species available that recapitulate the human immune response to the extent that the mouse does, and where the responses are understood to the extent that the mouse's is. Likewise, we cannot use less mature life stages as the mouse's immune system is not fully formed until after wean (around 4 weeks of age). We are modelling immune responses in adult humans, therefore we must use adult mice.
Lung function measurements, which involve exposing the trachea and mechanically ventilating the lungs, will be carried out under an anaesthesia from which the animal doesn’t recover so that the animal is not aware of what is happening. Apart from this procedure, all other procedures require too long a time to cause an effect to allow these to be carried out under terminal anaesthesia.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be carefully monitored throughout procedures, especially procedures which have the potential to cause harm. For instance, in parasitic infections, animals will be monitored very carefully during the period of infection when pathology may occur - the first 4 days of *N. brasiliensis* and *S. ratti* infection, and days 7-10 of *H. polygyrus* infection. In models of acute lung injury and asthma, peak illhealth can occur immediately after administration of allergens/chemicals, therefore animals will be carefully monitored at these times after administration, kept warm and supplied with supplemental oxygen if required, and culled if their health status does not improve.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidelines and webinars available at the NC3Rs website: https://www.nc3rs.org.uk/experimentaldesign

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through participating in the local animal users group, interaction with vets and NACWOs, and NC3Rs information that is shared around the department.

Home Office

NON-TECHNICAL SUMMARY

81.Immune responses in murine models of cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Immunotherapy, Cancer treatments, T cell responses, Checkpoint receptors, Biomarkers

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

1) To understand how immune responses and disease progression are altered by immunotherapies in different models of human cancer.

2) To identify markers that predict T cell responsiveness to immunotherapy.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This project seeks to explore how regulatory pathways activated during tolerance may also account for the exhaustion of immune responses in cancer. Given that most patients do not respond to immune checkpoint blockade therapy, there is an unmet need to understand the complex immunological consequences of checkpoint blockade. Furthermore, identifying early biomarkers of responders to these drugs would greatly aid in clinical management. For instance immune checkpoints "PD1" and "Lag3" are current targets for immunotherapy in cancers, however the majority of patients fail to respond to them. How they re-calibrate T cell activation thresholds and the exact phenotypic changes they impart are unclear. Thus, there is a need for greater insight into their effects on T cells and to identify biomarkers of successful responders. I aim to identify early T cell phenotypic biomarkers for responsivity to blockade of these pathways in vivo and gain insight into regulators of checkpoint expression. Thus, this work will not only lead to a better understanding of the mechanism of action of immunotherapies but will also identify priority biomarker candidates for future clinical studies.

What outputs do you think you will see at the end of this project?

We hope to identify a panel of markers that predict whether T cells are receptive to a given immunotherapy. These 'biomarkers' could then be used in future studies into human patient samples

We will generate new information on how different murine models of human cancer respond to immunotherapy, giving insight as to how these drugs work and how they might be further optimised in the future.

We envisage that this work will lead to several publications in reputable open access journals in the immunology/life sciences field.

We believe that the information generated over the project lifetime will support funding applications to begin human studies.

Who or what will benefit from these outputs, and how?

The project will deliver benefit to immunology and immunotherapy researchers in the short-term through the generation of new knowledge and understanding of important drugs (called immune checkpoint inhibitors) that are used to manipulate immune responses. It will identify similarities and differences between different immune regulatory pathways improving our understanding of the immune system and how to manipulate it.

In the medium term, the work here will be the first of its kind to perform a detailed investigation of how these different 'checkpoint inhibitors' work in distinct models of human cancer. Importantly, we will investigate responses to therapy in models of cancer that are either good or bad responders in the clinic to understand what may cause the differences immunologically.

In the longer-term, we envisage that the work may lead to the identification of biomarkers that can be used to decide whether a patient will respond to an immunotherapy thus helping to guide the treatment of cancer patients that receive immunotherapies.

How will you look to maximise the outputs of this work?

In order to maximise the outputs of this work we have identified two other groups within our establishment who work on similar models. These collaborations will enable sharing of expertise and optimisation of our experimental models. It will also maximise the chance of success of the project.

We will disseminate knowledge through publications, and for important data these may be released early into the public domain through non-peer reviewed pre-print servers such as bioRxiv.

We will promote our research through both internal and external presentations (e.g. seminars, conferences).

Species and numbers of animals expected to be used

- Mice: 3500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are used in these studies because: (i) the main components of their immune system are shared by humans; this is essential where immune responses as opposed to the function of individual genes is being studied. Other animals provide an inadequate model of the immune system; (ii) a wide range of wild type and genetically manipulated strains of defined genetic makeup are available; (iii) an extensive range of reagents is available, enabling the best possible analysis of

the cellular and molecular interactions occurring during immune responses. In addition, we are using a unique T cell activation monitoring system, which has been established in transgenic mouse lines.

We will typically use adult mice for experiments, since by then the immune system is fully developed.

Typically, what will be done to an animal used in your project?

We will study immune responses in several murine models of human cancer.

1) **Engrafted tumours:** in these models cells from tumours are injected under the skin of mice (called 'engraftment'). This will allow the establishment of a tumour to grow. We will then inject drugs that target immune checkpoints. We will monitor the tumour size in response to the treatment using non-invasive calipers. Drugs may be injected several times in order to optimise the therapeutic effect. Typically these experiments will last 2-6 weeks.

2) **Inflammation associated cancer model:** we will utilise a model of human colorectal cancer. In this model, mice receive injection of a drug that induces DNA damage. Mice then will be put on 2 weekly cycles on and off of a water containing an irritant that causes inflammation of the colon. By 8 weeks tumour development has occurred. We will then inject drugs that target immune checkpoints. We will monitor the tumour size in response to the treatment (through non-invasive imaging). Drugs may be injected several times in order to optimise the therapeutic effect. Typically these experiments will last 10-14 weeks.

3) **Spontaneous tumour model:** we will utilise a mouse model of human tumours (called familial adenomatous polyposis). These mice have a mutation in a gene important for regulating growth of intestinal cells. These mice spontaneously develop tumours around 6 weeks of age. We will then inject drugs that target immune checkpoints. We will monitor the tumour size in response to the treatment (through non-invasive imaging). Drugs may be injected several times in order to optimise the therapeutic effect. Typically these experiments will last 6-12 weeks.

What are the expected impacts and/or adverse effects for the animals during your project?

In all models tumours will develop, which can lead to weight loss, pain and changes in behaviour. Our protocols have been optimised to limit the adverse effects experience (e.g. preventing weight loss below 15% of starting weight). Tumours are typically present for between 2-10 weeks of the experiments.

In the inflammation associated cancer models, some animals may experience diarrhoea and rectal bleeding. This typically occurs during the on cycle of the water containing an intestinal irritant. Mice are carefully monitored and water containing intestinal irritants is removed when these signs occur, so these side effects are anticipated to be episodic and short lived.

For injections, mice will experience momentary pain, which is expected to be mild and short-lived.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

As these models lead to tumour development. It is therefore anticipated that the majority (two-thirds) of control mice will experience moderate severity. This reflects tumour burden and weight loss that may be induced. However it is anticipated that one third of mice who receive immunotherapies will likely experience milder symptoms.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Growth of tumours and the tumour microenvironment is highly complex, involving numerous cell types including the tumour cell itself, infiltrating immune cells, stromal cells and vasculature. There is also the biophysical properties of tumour masses to be considered, such as the fact that some tumour will be wholly or partially
anaerobic and poorly perfused with nutrients. Three mouse models of colorectal cancer will be used in this research. One is the engraftment model, this model allows us to test T-cell responses in tumour models that are known to provoke vigorous immune responses. This is important as a positive control. We will also use a model of human colitis-associated
cancer, and is induced by giving mice carcinogen (AOM) followed by repeated exposure to an intestinal irritant
(DSS) to induce colitis. The third model is the Apcmin/+ mouse model of familial adenomatous polyposis, which is caused by inheritance of a dysfunctional APC (adenomatous polyposis coli) gene. Mice lacking one copy of this gene develop multiple intestinal neoplasia. Both of these mouse models are considered to be highly contiguous with human colorectal cancer development and progression. Furthermore as human colorectal cancer is generally considered a poor responder to immunotherapy, we will be able to compare immune responses in both good and bad responder models. This would not be possible with any in vitro or in silico approaches.

Which non-animal alternatives did you consider for use in this project?

Organoids, in vitro and in silico methods.

Why were they not suitable?

Growth of tumours and the tumour microenvironment is highly complex, involving numerous cell types including the tumour cell itself, infiltrating immune cells, stromal cells and vasculature. Whilst organoids can form 3D structures, they are not wired to vasculature which is how immune cells enter and exit tumour environments. Therefore understanding the complex consequences of drugs that target immune cells within cancer would not be possible. Furthermore interactions between multiple

cell types as well as the 3- dimensional structure makes it very difficult to truly replicate in vitro or in silico.
We will work with collaborators to further explore the option of using Organoid models for pathways that we identify in vivo. We will employ in silico methods to refine our biomarker datasets by intersecting our markers with published ones in humans.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The project requires the breeding and maintenance of mice. In order to maintain colonies not all mice are subjected to further experimental procedures. We will utilise many different strains of mice to explore immune responses in cancer models. Typically we envisage breeding 500-700 mice per annum. This is based on us utilising 5-7 different mouse strains, with approximately one hundred of each strain used per year. Of these we envisage that up to 2000 will undergo procedures (the remaining 1500 mice are used to breed and maintain the colonies). This is based on 200 mice per year for engrafted tumour models, and 64 mice per year for colorectal and 64 mice for spontaneous tumour models. These are calculated based on 4 groups of 8 for each model being repeated on two independent occasions.

Pilot data suggest that n=5 is sufficient for engrafted models. However we will utilise more mice here to screen multiple checkpoint pathways, therefore we anticipate using more in this protocol.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have consulted with the NC3R's Experimental Design Assistant tool. This helps to design optimal experiments. Typically we aim to use what is known as a "randomised block design" which permits us to detect differences even when we introduce variables such as different sex and age. This approach controls for these differences by randomly assigning them to control and treatment groups. In this way we can maximise use of mice bred for the licence.

By using pilot experiments we will be able to determine the minimum number of mice to be used - typically we have determined that 6 mice per group will allow us to detect a reasonable treatment effect in most circumstances. However these calculations will be continually updated and improved throughout the life of the project.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

1. **Pilot experiments**

Small numbers of animals will be used in pilot experiments for each new type of study performed to assess intra- and inter-group variation. For most models in this project, optimal group sizes and variation are already well established by collaborators.

2. **Inbred strains**

The use of inbred strains (matched for age and gender) will reduce the amount of intra-group variation thus increasing the power of each experiment and reducing the group size needed to achieve statistical significance.

3. **Sensible breeding of transgenic lines**

Where possible mice will be bred as heterozygous x homozygous KO to enable usage of the whole litter.

4. **Multiple read-outs from same animal**

Multiple read-outs taken post-mortem from experimental animals will further reduce the number of animals required. For example, a single animal can be used for the analysis of bacterial CFU, activation of organ-resident cells/recruitment of peripheral immune cells (using large panels for multi-colour flow cytometry analysis), histopathological analyses, metabolic and cytokine profiling from frozen tissues.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

1: Engrafted tumour models

Engrafted tumour models require a single injection of tumour cells under the skin, minimising the pain and suffering mice experience. These models allow faster tumour development, tumours are visible, which allows easy non-invasive monitoring and ensuring tumours do not grow beyond 1.25cm3 in size.

2: Colitis associated colorectal model (CAC)

Azoxymethane (AOM) and Dextran sulphate sodium (DSS)-induced CAC is considered to be one of the most reliable models of human colitis-associated cancer. It has 100% incidence, which reduces animal numbers, while recapitulating disease progression seen in human from aberrant crypt formation, through polyp, adenoma
– adenocarcinoma development. Other CAC models that involve gene-deficient animal, usually coupled with an infectious trigger, are less reliable (varying from 20 – 80% penetrance), and

some involve severe immunocompromised animals which can increase pain, suffering and distress of animals.

The AOM/DSS model involves giving one injection of AOM. This induces DNA damage and DNA mutation. The day after AOM, DSS is given in the drinking water for 5 to 7 days. This induces colitis as DSS irritates the epithelial lining of the colon. Our collaborators within the establishment have refined the model and identified that 3% DSS is sufficient to induce tumour development whilst minimising adverse effects. Mice lose weight, develop diarrhoea and rectal bleeding, which resolves from 1 to 2 days following replacement with normal water. The DSS treatment is repeated twice more, after 2 weeks rest on normal water. Therefore by careful monitoring the suffering of mice can be minimised and symptoms eased through replacement with normal drinking water. Therefore whilst some unavoidable adverse effects occur we have made the above refinements to support the animals.

3: Spontaneous tumour model

We will utilise the APCmin/+ model, which is a model in which a gene important in regulating intestinal growth is deleted. This leads to spontaneous tumour development in the intestine. Therefore mice do not need to be administered substances such as carcinogens for tumour establishment, therefore minimising pain, suffering and distress.

Why can't you use animals that are less sentient?

This work has to be conducted in mammalian species, mice in our case, because the immune system, and therefore role in tumour development/treatment, in mammals is much more complex than that seen in invertebrates or other vertebrates. Hence, studying rudimentary immune systems will not lead to a significant increase in our understanding of the regulation of the immune system in health and disease. The mouse is the worldwide standard laboratory animal model and its immune system has been the most intensely investigated and for which there are the most reagents and information available. This, in addition to the availability of GA mouse stains, allows much more rapid progress to be made.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

CAC model

In terms of refining the model, we aim to use the least amount of intestinal irritant required and ideally aim for an initial
weight loss of not more 15%, although the humane endpoint is 20% we try and avoid reaching that, as a consistent 15% weight loss can still yield the desired results. We will achieve this by doing an initial pilot study to determine the sensitivity of mice to DSS, and use the lowest dose. We will be using a 4 point scoring system for assessing the severity of colitis in protocol 4. This takes weight loss, stool consistency, with a score of 0 being no effect and a score of 4 indicating the humane endpoint. We will utilise smallest gauge needle for administration and will explore water sweeteners for improving DSS palatability. We will follow LASA dosing guidelines.

Monitoring of mice

Mice will be typically monitored and weighed daily, therefore if any issues arise these can be dealt with as soon as possible to minimis mouse distress and discomfort.

Planning and revision

It is often not possible to always predict outcomes, and future experimental design is dependent upon the results of previous experiments and upon in vitro data derived from ongoing experiments. The data generated feeds back
into the experimental planning for improvement and refinement. Pilot experiments are often performed using small numbers of animals to assess variability and outcome. Thus, I will be continually revising the experimental plan and will continuously monitor the literature for refinements and the most up to date methodology to perform the outlined experiments.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will consult LASA guidelines on the handling and administration of substances.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly consult the NC3Rs website (www.nc3rs.org) and new staff will be directed to these web pages. Where new 3R advances occur we shall work with staff in our facility to adapt protocols or techniques. We will also seek advice and latest information through our Named Information Officer (NIO).

Home Office

NON-TECHNICAL SUMMARY

82. Immune responses to Marek’s Disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Marek's disease virus, lymphocytes, vaccine, chicken

Animal types	Life stages
chicken	embryo, neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to have a better understanding of immune responses to an infectious disease which causes cancer and suppression of immune response in chickens.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Marek's disease (MD), which causes cancer and suppression of immune responses in chickens, is associated with huge economic losses to the poultry industry and causes major welfare problems for the infected birds. As more virulent Marek's disease virus (MDV) are emerging, the control of the disease remains a major challenge to the industry. In this project, we will investigate the effectiveness of a novel form of vaccine "so called cell-free" and compare that with the conventional cell-associated vaccine. In the medium term, the results from this research will open a new avenue to develop more effective control measures. In the long term, this project should ultimately lead to control of virus and improved animal welfare and productivity in meat and eggs. The development of effective cell-free vaccines will remove the need for a cold chain for both storage and transportation of current conventional vaccines against this virus. Therefore, the development of a cell-free vaccine will reduce the cost associated with conventional vaccines for poultry farms and allow poor farmers in developing countries to have access to cheap vaccines.

The proposed project will also investigate the role of the immune response in the control of the disease in both resistant and susceptible chicken lines and identify those parts of viral proteins that can induce protective immune responses. Moreover, the results will provide information on whether infection impairs functions of immune cells which are important for the control of the disease in chickens. We will investigate and identify the factors released by infected cells or cancer cells which can impair the function of the immune response. Identification of novel biological pathways involved in causing the disease and the development of specific assays to study the immune response against Marek's disease virus are the short-term benefits of this study which could be used by other researchers in the field. However, in the long term, results could be used to design control strategies by blocking the effects of those factors released from infected or cancer cells which suppress immune responses.

Another important benefit is the development of tools and techniques to study functions of chicken immune cells which will be an important resource for other researchers to study immune responses to other viral or bacterial infections such as avian influenza virus, infectious Bronchitis virus and infectious bursa disease in chickens. Therefore, the benefits of this project could extend to improve the control of economically important infectious diseases in chickens.

What outputs do you think you will see at the end of this project?

- This work is expected to generate new information about the role of chicken immune cells in providing protection against cancer which is induced by Marek's disease virus. This information will be published in peer-reviewed scientific journals.

- Novel biological pathways, involved in suppression of immune response, are expected to be identified. This information will be published in peer-reviewed scientific journals.

- The effectiveness of a novel vaccine which does not require cold chain for storage and transportation will be examined. This information may lead to the development of novel vaccine product and the results will be published in peer-reviewed scientific journals.

Who or what will benefit from these outputs, and how?

Marek's disease, which causes cancer and suppression of immune response in chickens, is associated with huge economic loss (1-2 billion US dollars losses annually) to the poultry industry and causes major welfare problems for the infected birds. As more virulent Marek's disease virus (MDV) are emerging, the control of the disease remains a major challenge to the industry. In this project, we will investigate the effectiveness of a novel form of vaccine and compare that with conventional vaccine. In the medium term, the results from this research will open a new avenue to develop more effective control measures. In the long term, this project should ultimately lead to control of virus and improved animal welfare and productivity in meat and eggs. The development of the novel vaccine, which does not need cold chain for storage and transportation, will reduce the cost associated with vaccination and allow poor farmers in developing countries to have access to cheap vaccines.
The proposed project will also investigate the role of the immune response in the control of the disease and these results will provide information on how the virus can escape immune control. In the long term, results could be used to design control strategies by blocking the effects of the identified biological pathway to suppress immune responses.
Another important benefit is the development of tools and techniques to study functions of chicken immune cells which will be an important resource for other researchers to study immune responses to other viral or bacterial infections such as avian influenza virus, infectious Bronchitis virus and infectious bursa disease in chickens. Therefore, the benefits of this project could extend to improve the control of economically important infectious diseases in chickens.

How will you look to maximise the outputs of this work?

The results from this study will be published in peer-reviewed open-access scientific journals and presentations at scientific conferences and meetings.

Species and numbers of animals expected to be used

- Domestic fowl: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Marek's disease is an economically important disease in chickens, and thus chickens are the appropriate type of animals to study immune response to this disease. Vaccines against Marek's disease could be administered during embryonic stage or after hatching, and infection occurs after hatching.

Typically, what will be done to an animal used in your project?

Embryo and/or chicks will be vaccinated against the disease via various routes including subcutaneous and intra-abdominal routes and these birds may be re-vaccinated or infected with the virus at different time post vaccination.

Alternatively, unvaccinated chicks will be infected with the virus via different routes including intraabdominal or intra-tracheal routes and some of these birds may be administered with various substances, which modulate immune responses, via various routes including oral, intramuscular, intravenous and/or intra-abdominal routes. Blood samples or feathers will be isolated from the vaccinated/infected animals.
Duration of each experiment is approximately four months. Three different procedures used during the experiments are administration (e.g. subcutaneous, intra-abdominal, intramuscular, intravenous, oral), collection of blood samples from superficial veins and collection of feather samples.

What are the expected impacts and/or adverse effects for the animals during your project?

Injection causes a minor discomfort, and animals will recover very rapidly. Infection of unvaccinated chickens causes Marek's disease with clinical signs associated with formation of cancer and/or transient or permanent neurological signs. Some unvaccinated susceptible chickens may die from the infection without showing any obvious clinical signs or gross pathological lesions. The infected birds showing the clinical signs of the disease will be immediately culled. Infection of vaccinated chickens does not induce the disease, and the birds are protected from the disease.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Injection caused a minor discomfort, and animals will recover very rapidly (mild severity). Infection of unvaccinated chickens causes Marek's disease with clinical signs associated with Marek's disease (moderate severity). The infected birds showing the clinical disease will be immediately culled.

In approximately 75% of the animals which will be included in our experiments, we will use vaccinated chickens, or the birds will be administered with substances which increase the immune responses against the disease. Vaccinated chickens will be protected against the disease and they will show no clinical signs and they will not suffer from the disease. It is also expected that immune enhancing substances increase immune response against the disease and many of those birds will not show clinical signs or if the disease are induced a lower level of lesions are induced (moderate severity).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The project is aiming to investigate immune responses to Marek's disease virus, the causative agent of Marek's disease, an important viral disease in the chicken.

Which non-animal alternatives did you consider for use in this project?

Alternatives to animal procedures will be used in the proposed project where possible. For example, cell culture techniques will be used to assess the cytotoxicity of chemical inhibitors on chicken cell lines and primary cells for regulatory pathways.

Why were they not suitable?

The biological complexity of the immune system means that there is no alternative to the use of animals to study cell mediated immune response to virus infection and tumour development. Moreover, the efficacy of vaccines can only be tested in vivo and there are no in vitro or ex vivo alternative models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Based on our previous results and results published by other researchers, we have calculated the numbers of birds in each experimental group which can give us statistically significant results.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Advice was taken from statistician and we used online tools such as NC3R's Experimental design assistant

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Tissues will be collected and stored and shared with other projects.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In many experiments, we will increasingly infect vaccinated chickens or chickens with maternal antibodies, when it is possible. These birds will have some resistance to the disease.

Why can’t you use animals that are less sentient?

MDV only infects and causes disease in poultry, therefore it is not possible to use any other animals to investigate the immune response to MDV and develop improved disease control strategies

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In those birds that sudden death could occur after infection with the virus, the frequencies of inspection will increase to three times a day to identify any early clinical signs associated with the disease.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Published guidelines from NC3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will attend workshop, seminars on 3Rs and follow latest information on new development about 3Rs in online sources.

Home Office

NON-TECHNICAL SUMMARY

83. Immunity to ectoparasites of ruminants

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

vaccines, ectoparasite, livestock, ruminants, immunity

Animal types	Life stages
Sheep	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The specific aims of the project are:

1) To understand the nature of the protective immune response which is induced by exposure to, or administration of vaccines against, the ectoparasitic mite, Psoroptes ovis in sheep.

2) To use this information to improve the effectiveness of vaccines such that they will provide an important tool in the control of infestation with Psoroptes ovis (sheep scab or psoroptic mange).

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The vaccine that is being developed in the project will directly benefit livestock by protecting them from parasitic disease. This benefits both the welfare and productivity of the vaccinated animal but also reduces reliance on synthetic chemicals, adding to global food security and reducing environmental contamination. In addition, the parasite which is being investigated here has close relatives, which have negative health impacts on humans and other animals. i.e. scabies mites (Sarcoptes scabiei) and the house dust mite (Dermatophagoides farinae).

What outputs do you think you will see at the end of this project?

1) New prototype vaccine for further development to commercial products
2) New information on the host:parasite interaction
3) New information on vaccine and adjuvant effects on the immune system
4) Multiple scientific publications and lay articles on livestock vaccines

Who or what will benefit from these outputs, and how?

Short term beneficiaries will be researchers working in the fields of vaccinology and ruminant immunology through the availability of new information on the host:parasite interaction, immunology and vaccinology which will accelerate their own research programmes. Longer term beneficiaries include stakeholders in the vaccine and livestock industries through the production of new commercialisable tools for animal health and welfare. Societal benefits can also be seen through the reduction on reliance of injectable macrocyclic lactones and organophosphate dips with the attendant environmental and safety concerns that these bring.

How will you look to maximise the outputs of this work?

The applicant has a long track record of collaboration and effective knowledge exchange and outputs will be maximised through the following approaches:

Scientific Audiences: The results of our investigations will be published in open access journals appropriate for subject matter, 3Rs and scientific impact. All manuscripts will follow the ARRIVE guidelines [Kilkenny et al., 2010. PLoS Biol 8(6): e1000412] to promote accurate reporting appropriate for 3Rs initiatives.

During the course of the project we will attend scientific conferences (e.g. the World Association for the Advancement of Veterinary Parasitology Conference in Dublin, Republic of Ireland, 2021) to communicate progress and to emphasise the focus on scientific excellence in this project, highlighting crossovers in disciplines. The licence holder will attend the British Society for Parasitology Annual
Spring meeting each year to communicate these aspects. The licence holder is an active member of the BBSRC's International Veterinary Vaccinology Network and will attend their annual meetings to disseminate the approach and results (both successful and unsuccessful) to this community. The licence holder interacts with a large number of researchers through formal networks, which promote cooperation and multidisciplinary networking between scientists and stakeholders. In addition, as objectives are successfully completed we will directly communicate the emerging technology by email and/or Microsoft Teams meetings to the research groups most likely to benefit from it.

Educational and Public: The licence holder teaches undergraduate and postgraduate students in parasitology and will use these opportunities to promote the outputs and principles of this project. For dissemination to the wider public, the applicant will attend the Royal Highland Show in each year of the project, to communicate our results, their impact and context to members of the general public. The impact of the project will be made available on the host institution website as a resource for the public, policymakers and Government stakeholders in the food, animal health, environment and rural sectors.

Species and numbers of animals expected to be used

- Sheep: 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The animals used in these protocols are the natural hosts for the parasite under study. The life stages of the ruminants used are the most susceptible to this parasite and also the stages for which the economic impact of infestation is the highest.

Typically, what will be done to an animal used in your project?

Animals in this project will be administered vaccines by the most appropriate route (this may be injections or sprays for example) and then infested with Psoroptes ovis mites using our standard challenge infestation. Typically, animals would receive 3 injections of the vaccine, 2 weeks apart and would then be challenged with the mites for a maximum period of 6 weeks in experimental settings.

What are the expected impacts and/or adverse effects for the animals during your project?

Adverse effects on the animals are expected to be moderate. Infestation of the skin with Psoroptes ovis is characterized by exuberant yellowish scabs, additional signs include restlessness, pruritus, scratching, yellow-stained fleece, wool-loss, head tossing, bleeding wounds and loss of condition. In particular, the disease can cause considerable irritation or pain, or both. Typically, these effects would be for the duration of the infestation, so a maximum period of 6 weeks in an experimental setting. However, we have extensive experience on the course of infestation following experimental challenge and, at termination of the experiment the area of lesion is expected to be less than 20% of total body surface area. The injection of recombinant P. ovis proteins as part of the vaccine is considered unlikely to cause any adverse reaction, although animals will be carefully monitored during and immediately following these injections. Quil A adjuvant has been widely employed by ourselves and others in vaccines in sheep, without any adverse reaction. Infestation of sheep scab mites will be monitored closely.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All animals infested with *Psoroptes ovis* would be expected to suffer a maximum of a moderate degree of severity. This will be controlled by limiting the number of mites used for each application to ~100 mixed stage mites. Animals will be monitored, and infestations will only be allowed to proceed until a maximum of 20% of the body surface is affected or for a maximum of 6 weeks duration.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The parasites being studied in the project are "obligate" parasites. This means that they can only survive on a host animal. We must therefore use host animals (sheep) to maintain these parasites before and during vaccine trials.

Which non-animal alternatives did you consider for use in this project?

Currently there are none as the mite *Psoroptes ovis* cannot be maintained off of the live ovine host

Why were they not suitable?

Currently no suitable alternatives exist

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These estimates are based on the numbers of animals used in previous studies over the last ~10 years and have been optimised through collaboration with statisticians reviewing data from our previous experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Prior to each experiment, we review data from previous similar experiments, estimate the levels of effect of any treatment that we want to be able to record and collaborate with expert statisticians to determine the minimum numbers of animals required to accurately determine treatment/vaccine efficacy

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Maintaining good animal health throughout every experiment to avoid animal numbers reducing from disease which is not related to the disease under study. In addition, wherever possible we will make samples taken from live animals and any tissues from any euthanased animals available to other researchers for their use.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The species being used (sheep) is the natural host for Psoroptes ovis and also the target species for the vaccine being developed, so are the most appropriate model species to be employed here. Vaccination and challenge of sheep consists of 3 immunisations with the candidate antigen(s) in adjuvant (QuilA) two weeks apart and animals are then experimentally challenged with a carefully controlled mite infestation. During infestation and vaccine testing, sheep are routinely monitored by veterinary staff and are treated with veterinary medicines if required, based on clinical symptoms.

Why can't you use animals that are less sentient?

The mite, *Psoroptes ovis* has adapted to surviving on lambs and ewes and has a relatively long-lasting lifecycle (several weeks) and as such the use of terminally anaesthetised animals would not be practical. In addition, as previous infestations can result in a degree of protective immunity, we need to ensure that the animals used in the trials are naive for sheep scab with no history of infestation, which is easier to guarantee in younger animals with a known history.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Monitoring, post procedures, will identify any areas which require refinement. For example, animals are closely monitored following vaccination and, if any of the compounds administered cause pain, appropriate analgesia will be administered

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Where appropriate, the World Association for the Advancement of Veterinary Parasitology guidelines for best practise will be followed https://www.waavp.org. In addition, the ARRIVE guidelines 2.0 will be followed to allow the experiments to be performed in the most refined way and the publication of the data in the most appropriate form. https://arriveguidelines.org/

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The host Establishment has had involvement in numerous NC3R research initiatives and has regular contacts with this body. In addition, we have an active 3Rs committee, which provides advice both to project and personal licence holders and the local AWERB. Any relevant advances in the 3Rs will be implemented into any of the protocols where appropriate.

Home Office

NON-TECHNICAL SUMMARY

84. Immunity to ruminant endoparasites

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

vaccines, parasites, livestock, ruminants, immunity

Animal types	Life stages
Cattle	juvenile, adult
Sheep	juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The specific aims of the project are:

1) To understand the nature of the protective immune response which is induced by exposure to, or administration of vaccines against, parasites in ruminants.

2) To use this information to improve the effectiveness of vaccines such that they will provide an important tool in the control of production and welfare-limiting parasitic diseases of livestock.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The vaccines that are being developed in the project will directly benefit livestock by protecting them from parasitic disease. This benefits both the welfare and productivity of the vaccinated animal but also reduces reliance on synthetic chemicals, adding to global food security and reducing environmental contamination. In addition, the parasites which are being investigated here have close relatives which parasitise humans. The large-scale programmes which exist to produce vaccines against parasites of humans (e.g. the human hookworm vaccine initiative) may also benefit from the vaccine development and discovery work in this project.

What outputs do you think you will see at the end of this project?

1) New prototype vaccines for further development to commercial products
2) New information on host:parasite interactions
3) New information on vaccine and adjuvant effects on the immune system
4) Multiple scientific publications and lay articles on livestock vaccines

Who or what will benefit from these outputs, and how?

Short term beneficiaries will be researchers working in the fields of vaccinology and ruminant immunology through the availability of new information on host:parasite interactions, immunology and vaccinology which will accelerate their own research programmes. Longer term beneficiaries include stakeholders in the vaccine and livestock industries through the production of new commercial sable tools for animal health and welfare. Societal benefits can also be seen through the reduction on reliance of synthetic anthelmintics with the attendant environmental and safety concerns that these bring.

How will you look to maximise the outputs of this work?

We have a long track record of collaboration and effective knowledge exchange and outputs will be maximised through the following approaches:

Scientific Audiences: The results of our investigations will be published in open access journals appropriate for subject matter, 3Rs and scientific impact. All manuscripts will follow the ARRIVE guidelines [Kilkenny et al., 2010. PLoS Biol 8(6): e1000412] to promote accurate reporting appropriate for 3Rs initiatives.

During the course of the project we will attend scientific conferences (e.g. the World Association for the Advancement of Veterinary Parasitology Conference in Dublin, Republic of Ireland) to communicate progress and to emphasise the focus on scientific excellence in this project, highlighting crossovers in disciplines. The licence holder will attend the British Society for Parasitology Annual Spring meeting each year to communicate these aspects. The licence holder is an active member of the BBSRC's International Veterinary Vaccinology Network and will attend their annual meetings to disseminate the approach and results (both successful and unsuccessful) to this community. The licence holder interacts with a large number of researchers through formal networks which promote co-operation and multidisciplinary networking between scientists and stakeholders from EU member states. In addition, as objectives are successfully completed we will directly communicate the emerging technology by email and/or Microsoft Teams meetings to the research groups most likely to benefit from it.

Stakeholders and policymakers: The host Establishment has an established Communications Team through which we will attend the UK-wide Animal Health Roadshows each year to report on our progress and to promote the principles to both stakeholders and policymakers. The Licence holder will attend an annual Press Day in December each year to promote the findings of our project and to outline the benefits achievable with the outcomes. We have close interactions with many relevant stakeholders and lay updates on the research will be sent to these stakeholders through newsletters, website content and regular interactions at agricultural events and road shows.

Educational and Public: The licence holder teaches undergraduate veterinarians, scientists and public health students in parasitology and will use these opportunities to promote the outputs and principles of this project. For dissemination to the wider public, we will attend the Royal Highland Show in each year of the grant to communicate our results, their impact and context to members of the general public. The impact of the project will be made available on specific pages of the host Establishment website as a resource for the public, policymakers and Government stakeholders in the food, animal health, environment and rural sectors.

Species and numbers of animals expected to be used

- Cattle: 290
- Sheep: 1,130

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The animals used in these protocols are the natural hosts for the parasites under study. The life stages of the ruminants used are the most susceptible to the parasites and also the stages for which the economic impact of infection is the highest.

Typically, what will be done to an animal used in your project?

Animals in this project will be administered vaccines by the most appropriate route (this may be injections or sprays for example) and then infected with parasites which the animals would normally encounter in the field. Typically, animals would receive 2-3 injections of the vaccine, 3 weeks apart and would then be challenged with the parasites for 4-12 weeks in experimental settings, though these figures may increase in field experiments.

What are the expected impacts and/or adverse effects for the animals during your project?

Adverse effects on the animals may be loss of appetite, diarrhoea, reduced weight gain. Typically, these effects would be for 4-6 weeks.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Moderate severity: <10% of animals infected with gastrointestinal worms

Mild severity: ~90% of animals infested with gastrointestinal worms

What will happen to animals at the end of this project?

- Killed
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The parasites being studied in this project are "obligate" parasites. This means that they cannot survive outside of a host animal. We must therefore use host animals (sheep and cattle) to maintain these parasites before and during vaccine trials.

Which non-animal alternatives did you consider for use in this project?

Currently there are none.

Why were they not suitable?

There are none.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These estimates are based on the numbers of animals used in previous studies on previous versions of this licence and have been optimised through collaboration with statisticians reviewing data from our previous experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Prior to each experiment, we review data from previous similar experiments, estimate the levels of effect of any treatment that we want to be able to record and collaborate with expert statisticians to determine the minimum numbers of animals required to accurately determine treatment efficacy.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Maintaining good animal health throughout every experiment to avoid animal numbers reducing from disease which is not related to the disease under study. In addition, wherever possible we will make samples taken from live animals and any tissues from any euthanased animals available to other researchers for their use.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare

costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Cattle and sheep: gastrointestinal nematode infection models. Methods include blood and mucus sampling and faecal sampling, none of which cause anything more than transient pain or discomfort to the animal.

Why can’t you use animals that are less sentient?

These parasites are adapted to living in lambs, ewes, calves and cows and have relatively long-lasting lifecycles (several weeks) so the use of terminally anaesthetised animals would not be practical.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Monitoring, post procedures, will identify any areas which require refinement. For example, animals are closely monitored following vaccination and, if any of the compounds administered cause pain, appropriate analgesia will be administered.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Where appropriate, the World Association for the Advancement of Veterinary Parasitology guidelines for best practise will be followed https://www.waavp.org

In addition, the ARRIVE guidelines 2.0 will be followed to allow the experiments to be performed in the most refined way and the publication of the data in the most appropriate form. https://arriveguidelines.org/

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The project licence holder is the recipient of several NC3Rs grants and has regular contact with this funding body for reciprocal updates. in addition, the host Establishment has an active 3Rs committee which provides advice to both project and personal licence holders, the local AWERB and actively liaises with the Animal Technicians looking after the animals. Any relevant advances in the 3Rs will be implemented into any of the protocols where appropriate.

Home Office

NON-TECHNICAL SUMMARY

85. Impacts of diet or supplementation on rumen function

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words rumen, microbiome, feeds,

supplements

Animal types	Life stages
Cattle	adult
Sheep	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project will determine the impacts of diet or supplements on rumen function in cattle to help improve their overall health, welfare and productivity.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Farm animals can be affected by a whole manner of diseases that can be related to diet, and they can have a significant impact on the animal's health and wellbeing, in addition to its production characteristics such as milk production. By investigating the effects of diets or supplements or rumen digestion, metabolism, the rumen (the fermentation chamber of the ruminant gut that degrades fibre) microbiome (the beneficial microbes that live in the gut) and rumen function, it will be possible to develop new feeding or supplementation strategies that will maintain or enhance animal productivity, while not compromising animal health or welfare, and in fact should hopefully improve animal health and welfare. As part of these studies we may use rumen instrumentation boluses (electronic devices that reside in the rumen that transmit data to a computer) to gather real-time data on rumen pH and motility to further enhance our understanding of the effects of diet or supplementation on rumen function.

What outputs do you think you will see at the end of this project?

The outputs at the end of this project should be improved feeding or supplementation strategies that should maintain or improve dairy production parameters (e.g. reduction in incidence of metabolic disease), and importantly rumen health and overall animal health and welfare status should be enhanced. Given the financial impact of metabolic disease on the dairy industry, reducing the incidence of these diseases via novel feeding or supplementation strategies will be of considerable benefit. It is anticipated that by the end of this project we will be able to develop guidance or fact-sheets for dissemination to farmer groups/organisations for extension and knowledge transfer purposes.

Academic outputs will also include peer-reviewed publications, undergraduate or postgraduate theses.

Who or what will benefit from these outputs, and how?

These studies are directly applicable to cattle being maintained in typical production systems, where improved feeding or supplementation strategies will maintain or improve animal productivity, without compromising animal health, wellbeing or welfare.

More specifically:

- Individual animals or an individual herd may benefit from a reduction in the incidence of metabolic diseases through our development of novel feeding or supplementation strategies.
- From a business perspective farm businesses and the wider industry should see a reduction in the financial cost to the business from metabolic diseases.
- Adoption of novel feeding strategies with positive outcomes of lower disease incidence will lead to more efficient (nutritional and financial) management of the animal/herd, improving animal productivity and gross margin outcomes.
- Gaining a better understanding of the microbiome population and how it dynamically changes in response to diet, feed processing (e.g. rolled grains) or supplementation strategies will allow us to consider how best to 'support' the beneficial microbes within the gut via these strategies in order to help maintain a more stable microbial population that meets the challenges of rapid dietary changes that occur when animals are going from the dry period into peak lactation, into late lactation. Furthermore, we are aware of how the quality of milk changes throughout the season (spring grass versus summer/autumn grass), and again our studies in this project on the microbes within the gut will lead to further studies in the medium to longer term determining how fluctuations in milk composition could be reduced through a better understanding of the role that gut microbes play in rumen function.

How will you look to maximise the outputs of this work?

In addition to disseminating to the wider academic community through peer-review papers etc., we aim to disseminate the knowledge obtained from this project to the wider farming community via their community organisations, knowledge transfer events and social media. For example, we may be able to represent our results via seminars at various agricultural shows or similar farming events. We also aim to set up webinars later in the project to update farmer groups (e.g. DairyCo, young farmers clubs) on the dietary or supplementation strategies we've developed that may improve animal productivity, efficiency, health and welfare of their animals.

Species and numbers of animals expected to be used

- Cattle: 18

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

These studies are relevant to commercially reared and maintained cattle, therefore it is entirely appropriate to use adult animals of these species for these studies.

Typically, what will be done to an animal used in your project?

For animals in this project they will be subjected to surgical modification through the creation of a permanent rumen fistula, which is the creation of a hole in the side of the cow to allow direct access to the rumen. A precise cannula is fitted in to this area, which is in the form of a specially fitted covering with a removable plug, to keep it maintained and to keep oxygen out of the rumen. This modification is essentially the same as that created in human patients with a stoma. Having an opening into the rumen from the outside is essential for us to monitor in real-time fluctuations in pH, digestion, metabolism and gut microbes in response to diet or supplement administration. This principally involves sampling digesta, faeces, or taking peripheral blood samples or samples of the lining of the gut. Rumen-fistulated animals can remain healthy for many years, and it is anticipated that on average these animals often live for over 10 years with the permanent fistula, which is much longer than a 'typical' commercial animal.

What are the expected impacts and/or adverse effects for the animals during your project?

The main impacts with creating an artificial opening into the gut of cattle is mild to moderate pain and discomfort for a brief period of time following surgery. Once animals have fully recovered from the surgery then tend to have minimal discomfort and can lead long lives on these studies. All other procedures used in this project are classified as having mild and transient adverse effects (e.g. from blood sampling).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of cattle undergoing this surgery will experience moderate pain in the immediate post-operative period, but this is transient and resolves over a few days typically. Animals are permitted at least 6 weeks to fully recover to ensure there is no pain or discomfort during their use for subsequent nutritional studies.

All other procedures in cattle are a mild severity for a transient period during sampling.

What will happen to animals at the end of this project?

- Killed
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of live animals is necessary as it is not possible to model in laboratory conditions the complex nature of rumen function, rumen contractions, feed interactions, gut microbes and effects of the production cycle, such as pregnancy or lactation.

Which non-animal alternatives did you consider for use in this project?

There are systems and equipment available in the laboratory that can simulate the rumen of cattle by investigating the rate and extent of how feeds are digested through measuring how much fermentation is going on, which is the process by which beneficial rumen microbes degrade fibre.

For the within animal inter-batch testing of rumen vitamin and mineral boluses (devices that reside in the rumen and slowly release vitamins and minerals) we aim to develop a parallel programme of work to develop a novel laboratory process that can mimic, to an extent, digestion in the gut of cattle, such that ultimately animal testing may not be required in the future.

Why were they not suitable?

While the laboratory systems can give some insights into feed digestibility by measure fermentation as a proxy measure, it does not adequately model the complexity of rumen function, and cannot simulate rumination (chewing the cud) or the complexities of the microbial community in the rumen compared to the live animal.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers have been estimated based on over 30 years of experience conducting these studies, and by the number of studies planned over the lifetime of this project. It also includes the animals to be transferred from the previous PPL. Experiments will be designed taking into account biological

variation of parameters to be tested, in order to use the minimum number of animals required to establish statistically significant outcomes.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The use of surgically-modified cattle for these studies vastly reduces the numbers of animals required for these studies as a result of them being able to be reused, including through utilising experimental designs such as cross-over studies. If we were to use 'normal' cattle then there would be a marked increase (in to the hundreds) in the numbers required to fulfil the work on this project. Their use is well validated in the literature. In addition, the continued use of surgically-modified animals for another protocol on rumen function studies in this project also minimises the overall number of animals required.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Where appropriate these studies we will utilise pilot studies to initially 'screen' diets or supplements that are to be taken forward to a full experiment. To a large extent pilot/screening studies will be completed in the laboratory using the simulated gut equipment to investigate digestion. This will allow us to screen a large number of feedstuffs (including those developed using novel processing techniques) and/or dietary supplements (e.g. probiotics) to see initially how they affect digestion. Only those feedstuffs or supplements that appear to significantly affect digestion parameters in the laboratory will be selected for further study in the animals. Together, these measures will optimise and minimise the number of animals utilised in this project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In this project we will surgically prepare cattle to have a rumen fistula. The rumen is the largest part of the digestive system in cattle (functionally similar to the human colon) and it is where fibrous foods are degraded by a complex microbial community living in the rumen. The creation of a rumen fistula is very similar to the creation of a stoma in human patients who require it due to various diseases, such as bowel cancer or Crohn's disease. Cattle who get this surgery cope very well and this type of surgical modification has been used extensively since the 1950s, hence we are very well aware of how cattle live with this modification. It does not require a general anaesthetic and following initial periods of recovery with painkillers, these cattle can go on to live a very long life with this fistula without the need for painkillers, and in most cases these cattle are able to live much longer lives in relative luxury in comparison to commercial cattle. In order to minimise any harms in the medium to longer term, the

cattle undergo a strict regime of health monitoring and maintenance/cleaning of the opening to the gut. For example, in addition to daily health care assessment, once cattle are fully recovered from their surgery we physically monitor their fistula site regularly (at least weekly) for signs of irritation or ulceration. In addition, the cannula we use to maintain the access point to the gut is replaced at least annually, or as soon as needs be if upon inspection the material becomes brittle. We use an objective scoring system to ensure the overall animal's health status is maintained, and their fistula site is healthy.

From a scientific standpoint this model is entirely appropriate as it allows the frequent access required to the rumen to complete the objectives of individual experiments, such as inserting small porous bags containing test feed or supplement materials or instrumentation (data-transmitting) boluses. It is not possible to use normal animals and pass feed materials via stomach tube for these types of study, as we need to recover these feed materials at time points over the course of the study, and instrumentation boluses at the end of a study, and this would not be possible by stomach tube. If we used normal animals without a fistula we would have to use vastly greater numbers and slaughter them at the time points throughout different studies.

Why can’t you use animals that are less sentient?

These studies are aimed at examining gut function and microbiome parameters in adult dairy cattle, therefore we are required to use adult animals in these studies. There are no less sentient ruminant species, hence why adult cattle are required.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animal suffering will be minimised by using experienced licensees for all procedures. In addition, feed products, supplements or boluses are produced under strict regulatory frameworks and are not anticipated to have any adverse effects as most are already available on the open market. Animals are managed by trained personnel to ensure they are managed and trained/habituated to the sampling procedure process. For rumen fistulation surgery we have well developed protocols in place for the procedure itself, plus the post-surgical monitoring of the animal and fistulation site. This includes pain management during the procedure and post-procedure. We also have protocols for continued monitoring and scoring (lesions) of the fistula site, with inspections formally undertaken weekly and monthly. Following recovery from surgery, the cannula, which is the covering that sits within the site of the fistula to keep it clean and to ensure oxygen does not get into the rumen, is changed at least annually, or beforehand if the material becomes brittle.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

For the studies included in this project we will follow published best practice as laid out in PREPARE (published by Norecopa, a Norwegian platform for the advancement of the 3Rs) and ARRIVE (published by the UK's NC3Rs) guidelines. When conducting scientific studies within this project we

will utilise and be conscious of the principles laid out in the PREPARE guidelines, to ensure that the experiment is designed and conducted in a manner that is scientifically valid and reproducible. The ARRIVE guidelines are a complementary set of guidelines to PREPARE that will be used to inform us of best practice in the preparation of reporting the outcomes of this project for dissemination/publication to scientific or other groups (e.g. farming community groups).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I am a member of AWERB, and routinely attend events/meetings organised by the establishment specifically focused on the 3Rs. This culminates in an annual group meeting of staff involved in ASPA activities where we review the year's activity and discuss examples of best practice in relation to the 3Rs. Furthermore, I collaborate with colleagues nationally and internationally who also have fistulated animals and we share resources and protocols (including health monitoring protocols) as necessary.

Home Office

NON-TECHNICAL SUMMARY

86. Implications of therapeutic inhibition of the complement system on infectious and noninfectious inflammatory diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Complement System, Kidney injury, Bacterial infection, Fungal infection, Immunotherapy

Animal types	Life stages
Mice	adult, juvenile, neonate, pregnant, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

This project aims to validate the utility and efficacy of a novel therapeutic technology to reduce kidney injury in some diseases. We will also look for possible side effects that may result from using this technology like an increase in the risk of infection with microbial pathogens and how to reduce this risk.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

The complement system (a group of circulating proteins in the blood) is an important component of the immune system that participates in recognition and killing of invading microbial organisms. Activation of the complement system also triggers the inflammatory response. Uncontrolled activation can cause tissue injury and the development of some inflammatory diseases. Development of inhibitors that block the complement system in the blood will open a new avenue for novel approaches and strategies for the treatment of inflammatory disorders. Unfortunately, the use of complement inhibitors may increase the risk of microbial infection in patients and hence it is important to address the fundamental interactions between the host immune system and different pathogens during the course of active infection. Understanding the underlying mechanisms and pathways involved in the immune defence will also help to develop novel approaches for the treatment of bacterial and fungal infections.

What outputs do you think you will see at the end of this project?

Our research in the next five years will provide novel findings that will help to identify novel therapeutic approaches that will help to alleviate complement-mediated tissue injury especially in case of haemolytic uremic syndrome (HUS) and lupus nephritis. In addition, our results will provide a better understanding of how the immune system behaves towards invading pathogens especially in case of complement deficiency. This will help to develop new strategies for the treatment of bacterial and fungal infection. We always publish our new findings in high impact factor scientific journals to disseminate it to the scientific community. Unsuccessful approaches will also be highlighted in our publications and discussed with our collaborators.

Who or what will benefit from these outputs, and how?

In the short term the outputs will be communicated to academics working in the field of immunology, where we will assess both the benefits and the harmful effects results from complement activation and address the delicate balance between complement activation and down-regulation in health and disease.
In the long term we will introduce a new and well-defined study in the field of complement immunology during infection that will help to better understand how the immune system fights microbes, and how to avoid the harmful effects resulting from uncontrolled activation of the immune system. This work might also introduce new therapeutic approaches that will help to reduce kidney injury in lupus nephritis and HUS and improve the outcome in these patients.

How will you look to maximise the outputs of this work?

Our group has a close collaboration with experienced Senior Scientists in our field, who have established track records in mouse models of experimentally induced infectious disease, stretching over several decades whom I meet on a regular basis to discuss results and future experiments. A complete survey on the published data has already been done and we know exactly what is published in our area of study. We will use the most refined and the most reliable models for our infection studies as well as for HUS and lupus nephritis models. We always publish our new findings in high impact factor scientific journals to introduce it to the scientific community. Unsuccessful approaches will also be discussed with our collaborators and will be mentioned in our publications. Sharing of animal tissue from our well-designed experiment with our collaborators will help to maximise the benefits.

Species and numbers of animals expected to be used

- Mice: 2000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use adult wild type and adult complement deficient mice that lack one or more of the essential components of the immune system. Using these mice will give a clear overview of how the absence of these immune components will affect the infectivity and pathogenicity of different pathogens included in this project. We will also use adult MRL-lpr/lpr (Lupus) mouse, a strain that is known to spontaneously develop lupus nephritis and kidney injury after 14 weeks of birth. This model will help us to validate how inhibition of the complement system will improve kidney function in such mouse model.
We will use adult mice in our models of diseases because the immune response at that stage captures the essential traits of many bacterial infections and tissue injury in humans.
Typically, what will be done to an animal used in your project?

In our mouse models of microbial infections:

- Mice will be infected with bacteria or fungi via intranasal or oral administration as well as by injection.
- Mice will be observed for disease progression.
- Immunomodulatory and/or complement inhibitors therapeutics could be injected at any time either before or after the infection.

- Blood samples will be taken at different time points post-infection to assess the bacterial load in blood to avoid any complications that may increase the severity of disease progression beyond the expected severity limit.

- Our experience shows that the infection study in protocol 1 will last for 3 weeks while other infection models in protocol 2 will last for 7 to 14 days.

In case of lupus nephritis model:

- Mice at the age of 14 weeks will start to develop signs and symptoms of renal injury.
  At that age we will treat mice with immunomodulatory and/or complement therapeutics that will reduce
- complement-mediated kidney injury.

- In some experiments, mice will be treated with complement inhibitors therapeutics/immunomodulatory compounds at earlier ages (10 -12 weeks).

- This study will continue for 8 to 10 weeks

All animal experiments will be randomised and people who are assessing the animal will not be aware of whether they are assessing control or treated mice.

What are the expected impacts and/or adverse effects for the animals during your project?

In mouse models of infection, mice will show signs and symptoms of moderate severity limit including reduction in their activity within the cage and decrease in body weight. Animals will be closely observed as frequently as necessary and at least every 6 hours to ensure that these symptoms would not last for more than 12 hours. If these signs persist for over 12 hours or if an animal begins to deteriorate at any time, it will be immediately killed.
Lupus nephritis is inflammation of the kidney due to an auto-immune disease known as systemic lupus erythematous. With lupus, the body's immune system targets its own body tissues including kidney causing kidney inflammation and kidney injury. In a mouse model of lupus nephritis, mice will start to show some complications depending on the stage of the disease progression and the age of the mouse. From 14-16 weeks old, mice will show symptoms of mild disease severity such as a decrease in body weight (5-10%) and a slight increase in some biological markers that reflect how kidneys are functioning such as blood urea nitrogen (BUN) and creatinine levels in the blood. Older mice will show a moderate decrease in body weight (10-15%) and significantly elevated levels of BUN and creatinine levels.
Becuase the strain of mice that we will use in this protocol spontaneously develop auto-immune disorders, mice will also show some other side effects such as enlarged lymph nodes and skin lesions that will be considered during the course of our experiment.
Animals will be humanely killed by once they reach predetermined end-point for this protocol.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

1. In protocols 1 we will use 500 adult mice at moderate severity limit
2. In protocols 2 we will use 1000 adult mice at moderate severity limit
3. In protocols 3 we will use 500 adult mice at moderate severity limit
4. In protocol 4 we are expecting mild severity limit (All animal).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We will study how the immune response mediates tissue injury in some auto-immune diseases such as Haemolytic Uremic Syndrome (HUS) and lupus nephritis. In addition, we need to study the complex interaction between the innate immune response and different pathogens. These processes involve a complex interaction between immune cells, plasma proteins, the blood vessel walls (vascular endothelium) and organ-specific cells that cannot be modelled using ex vivo (using animal tissues in cell culture) or in vitro (in test tubes rather than in animals) systems.

Which non-animal alternatives did you consider for use in this project?

Prior to animal experimentation extensive in vitro studies using different techniques to assess the ability of different proteins from the immune system to bind bacteria such as (ELISA and FACS analysis techniques). Opsonophagocytosis assays (engulfment of bacteria by white blood cells isolated from human blood) will be performed to identify which strains/serotypes of the pathogen will stimulate the targeted pathway of the complement system. Assessment of the functional activities of the complement inhibitors will be completed first in-vitro using ELISA and FACS analysis to evaluate the specificity and efficacy of these complement inhibitors. We considered the possibility of limiting the infection experiments to ex-vivo studies using blood from human volunteers as a source of complement and cells. Ex-vivo studies do not reproduce the course of the infection in multi-organ model (i.e. in animals)

Why were they not suitable?

It is necessary to study microbial interaction with the immune system in a whole mammalian organism to appreciate the impact of medical treatments on:

a) The spread of the bacteria between different organs in the body

b) Tissue pathology in various organs

c) The effects of the progressive escalation of immune responses

In addition, we need to assess the degree of kidney injury as a result of uncontrolled complement activation in some autoimmune disorders such as lupus nephritis and HUS. This study requires the tissue to be exposed to different complement components and recognition molecules for a prolonged period of time to achieve tissue injury and there is no available ex-vivo model for such complications.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our long-term experience in animal work, using good experimental design helped us to give the estimated numbers of animals that we will need to use in each experiment. We also consulted with experienced scientists in our field and within our group. In addition, we consulted a departmental statistician who gave us advice and help whenever needed. Our previously similar animal models published in high impact factor scientific journals were also a useful guide to estimate the number of animals that will be used in this project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Whenever possible we perform preliminary studies using smaller numbers of animals. We also try to adjust our work to use only one control group that can be used for different experiments. We use the smallest possible experimental number of animals for each experiment being very careful that this does not affect the accuracy of the results. To calculate the smallest number of animals that we can use, calculations based on advanced statistics and mathematics in addition to other published results were taken into considerations. Online tools such as experimental design assistant (EDA) from NC3Rs website were used to perform sample size calculations. To increase the quality, reproducibility and translatability of our animal studies we will follow PREPARE guidelines (Adrian et al., 2018). Animal tissues from different experiments will be kept frozen and/or embedded in paraffin blocks that can be used later if more tissue analysis is required.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We always run pilot experiments using a small number of animals to assess the feasibility of the study.
In some experiments we will use bacterial toxins instead of using the bacteria. This will help to minimise the side effects where mice will recieve only the calculated dose of the bacterial toxins that show the symptoms and the signs of disease without exposing animals to the bacteria. We will run pilot experiments to test the potency of the bacterial toxins and so large stock of bacterial toxins will be purchased to avoid repeating this step every time we buy a new batch.
We would not start any animal work in large groups until we have preliminary positive and encouraging data.
Tissues from pilot experiments and major experiments will be kept frozen or fixed in paraffin blocks for future use. For genetically modified mice, we will follow a breeding protocol that gives us only our gene-targeted mice by breeding deficient mice whenever possible to avoid the production of unnecessary mice.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs

(harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

All animal models in this study are designed to cause less pain, stress and suffering. All the necessary procedures will be taken to achieve high levels of welfare to the animal starting from good husbandry and animal care before starting the experimental procedures. We will use the most refined mouse model of infection for each pathogen. During the experimental procedures in the infection study (Protocols 1 and 2) all requirements will be taken to cause less stress and minimal harm to the animal. e.g. In case of i.n (intra-nasal) infection we will use light anaesthesia to reduce animal stress and maximise the volume being delivered. We will use small gauge and fine needles to minimise trauma in the case of intra-venous and intra-peritoneal drug administration. Mice will be monitored for any unexpected signs and symptoms of disease progression to avoid exceeding the allowed severity limit. Mice will be euthanised at early end points to avoid exceeding the allowed severity limit (moderate).
In the lupus nephritis (kidney injury) model (Protocol 3), we will use a specific strain of mice that spontaneously develop kidney disorders and validate our complement inhibitor therapeutics to prevent the onset of disease progression. Lupus prone mice will survive normally without any complications until they reach the age of 14 weeks when they start to develop signs and symptoms of lupus nephritis and kidney injury. These symptoms include; elevated levels of creatinine and blood urea nitrogen, increased albumin and protein levels in urine. We will not inject any compounds that could accelerate kidney injury but ameliorate or prevent inflammation and tissue injury. We will use therapeutic compounds that are expected to minimise the kidney injury and improve the renal function.

Why can’t you use animals that are less sentient?

We need to study the immune response towards bacterial infection and how the immune response might cause tissue injury as a result of uncontrolled activation of the complement system. This will require animals to be exposed to the insults for a relatively long time (days or weeks) and this cannot be done in anaesthetised mice. In addition, using immature life stage of animals is not a good choice to study the immune response, especially since immature developmental stages of animals are likely to have an immature immune system and therefore will provide results that cannot be extrapolated to reflect the responses expected in mature animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Mice will be closely monitored for any sign of disease progression. Mash (soaked mouse pellets which are easier to access and eat) will be used during our studies if needed to minimise weight loss. Any pain or stress will be diagnosed as early as possible and animals will be culled by humane methods (schedule 1 methods) if necessary. A proper environment including shelter and a comfortable resting area will be provided to the animals. All clinical signs and manifestations will be scored in scoring sheets. These scoring sheets will be kept and discussed after each experiment to refine our procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Our group has an excellent reputation in the field of complement immunology and we keep ourselves updated with all new and novel experimental designs and models in our field. This includes non-animal models. All newly published data in our field are discussed internally within our group to enrich our knowledge and that could help to find novel experimental designs or other non-animal models that could help in our study. In addition, all our experiments will be conducted in a way that allows us to publish our results according to the ARRIVE guidelines. we will follow all guidelines provided by LASA,
PREPARE and NC3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By following the updates on NC3Rs and NORECOPA (websites as well as additional guidance literature from Laboratory Animal Science Association (LASA). Related events such as conference and symposium will be attended.

Home Office

NON-TECHNICAL SUMMARY

87. Improved targeting of cancer for imaging and therapy

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Cancer, Imaging, Therapy, Theranostics

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to develop approximately 4 novel imaging agents, 1-2 drugs for cancer therapy and 2-3 theranostic agents.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The proposed research will accelerate the development and clinical translation of new imaging agents and cancer therapies. The research will:

i. Enhance the understanding of cancer biology in living subjects.

ii. Develop new imaging agents for cancer.

iii. Develop new methods to indicate very early on whether patients are not responding to treatment, avoiding unnecessary toxic side effects for patients, saving time and treatment-associated costs, and providing an opportunity for patients to be switched to other drugs.

iv. Develop new targeted therapies that exploit tumour biology, including 'imaging-directed targetedagents' for selective killing of cancer cells or reversing of drug resistance with minimal toxicity.

Our research group is multidisciplinary and includes a clinical team of medical doctors that are able to translate compounds that are successfully developed preclinically into the clinical setting by performing clinical trials in humans. Thus, there is potential for direct impact to cancer patients.

What outputs do you think you will see at the end of this project?

The main outputs of this project are:
- Development of new imaging agents (~3 compounds in 5 years) - i.e., contrast agents that accumulate specifically in the tumour providing better imaging contrast - that are successful enough to progress for use in humans.

- Development of new therapeutic agents (~1-2 compounds in 5 years) - i.e., compounds that can be used for treatment of tumours - that are successful enough to progress to clinical trials.

- Development of new theranostic agents (~2 compound in 5 years) - i.e., compounds that allow simultaneous diagnosis (imaging) and treatment (therapy) of tumours - that are successful enough to progress to clinical trials.

- Understanding the biology of cancer in living subjects;

- Publications to increase the existing knowledge about cancer.

Who or what will benefit from these outputs, and how?

The outputs of this project will primarily benefit cancer patients. We will develop imaging agents that allow earlier diagnosis and staging of cancer non-invasively, instead of being restricted to biopsy, an invasive procedure that is often not accurate at representing the whole tumour, and more difficult to use in the metastatic setting. Our imaging agents will also help predict which patients/tumours are likely to respond well to certain treatments. Furthermore, these agents can be used to evaluate the success of the treatment very early on, thus saving patients from the suffering caused by side effects of treatments and offering the possibility of switching to more beneficial treatments before the tumour becomes too aggressive. We will also develop cancer treatments that have maximal anti-tumour activity while minimising side effects to the rest of the body, and that prevent or reverse resistance to drugs. Lastly, we will develop theranostic agents - i.e. compounds that act as both imaging and therapy agents - which will allow treatment and its monitoring simultaneously, contributing to the likelihood of treatment success.

How will you look to maximise the outputs of this work?

We will aim to maximise the outputs of this project by seeking collaborations with appropriate expertise whenever suitable; by bringing the whole group (and possible collaborations) together to think about experimental designs or troubleshooting possible arising issues; by sharing the knowledge with the community and by publishing all results, whether positive or negative.

Species and numbers of animals expected to be used

- Mice: 7400
- Rats: 590

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Our aims are to develop new imaging agents, new targeted drugs and new theranostics (where imaging and therapy are combined in the same molecule) for personalising cancer care. The need for this is that 1 in 2 people will be affected by the disease in their lifetime making efforts to develop these diagnostics and therapeutics important; particularly because current drugs are ineffective in most cancers. A significant part of our work will be done using isolated cancer cells grown in the laboratory in cell culture dishes, as well as computer-based approaches. These initial experiments will ascertain relevance of the processes being studied, and permit optimisation.
Ultimately, however, animals have to be used to determine whether in the whole organism, the specific biological target is being modulated, and if the sensitivity and specificity or contrast are achieved over healthy organs. Our approach is staged: starting from animal models that are easy to monitor visually to more complex models for which we will add imaging to clinical signs for monitoring. Mice and rats will be used in the studies because they are the simpler animal for which suitable models of cancer are available. Adult mice – usually 6-12 weeks - will be used. This is because mature rodents have more stable physiological state (respiratory/pulse rate, rectal temperature, weight) and are more tolerant to infection. In some cases, will use immune-deficient animals for tumour induction as this will give us the opportunity to study the biology of human tumours without

rejection; the studies will be done in a manner to avoid infection.
Most animals will undergo procedures involving mild discomfort like a pin-prick and non-invasive imaging. Animals will be anaesthetised for the imaging procedure. Animals may receive therapeutics, undergo surgery or develop tumours, procedures that could potentially cause moderate discomfort. Where appropriate, animals will be given analgesia/anaesthesia to avoid pain. Animals will be monitored closely including use of image-guided monitoring of tumour size. Serial sampling by imaging is both better science and reduces animal use; it also provide earlier disease detection than clinical signs, thus less severe. Animals will be housed with appropriate bedding, nestling material and with cage toys for a stimulating cage environment. We will monitor animals in accordance with National Cancer Research Institute (NCRI) guidelines for the welfare of animals in experimental neoplasia, as well as UK Laboratory Animal Science Association good practice guidelines (LASA guidance). At the end of the study, animals will be humanely killed or euthanised. In summary, we aim to develop future imaging diagnostics and novel therapeutics that will benefit science, animals and people, without compromising animal welfare. We consider the overall severity as moderate.

Typically, what will be done to an animal used in your project?

Typically, an animal used in our project will be given a tumour though injection of cancer cells under the skin, although in some occasions we may inject them in the organ where the tumour normally grows (i.e., a mammary tumour will be injected in the mammary fat pad of an animal). The animal and its tumour will be monitored until the tumour is big enough to undergo experimental evaluation - this usually happens 3-4 weeks after injection of cancer cells. Experimental evaluation involves giving the animal a new compound that has been developed in our lab, which may fall into three classes: therapeutic agents, i.e., compounds with anti-tumour activity for cancer therapy, imaging agents, i.e., contrast agents that allow visualisation of the tumour through imaging, or theranostic agents, i.e., compounds that allow diagnosis and therapy of cancer simultaneously. For example, for evaluation of an imaging agent, the animal will be anaesthetised, have the contrast agent injected in its tail and put inside a temperature-controlled imaging scanner for 1 hour. Once the scan is finished, the animals will be humanely killed while still under anaethesia so that the tumour tissues can be collected to validate the experiment and confirm the accumulation of the compound tested.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the animals used in our project will be implanted with tumours under the skin which won't cause significant discomfort because the tumours will be of relatively small size. However, in some cases, where the tumours are grown in their organ of origin - say, a prostate cancer is grown in the prostate - the animals may experience adverse effects associated with tumour growth. These are vary depending on the tumour type, (for example, prostate cancer may lead to changes in urinary flow, whereas brain cancer may affect the animal's behaviour) and, depending on the level of pain and distress that causes to the animal, we may either treat these symptoms or kill the animal to prevent further suffering. Furthermore, while we aim to design safe compounds, they may, sometimes, lead to side effects such as loss of appetite or alterations in behaviour. Once again, these symptoms may be treated or the animal may be killed, depending on the extent of the symptoms. Clear limits have been outlined in this project licence that will guide the decision to either treat or humanely kill an animal.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In this project, 50% of mice are likely to experience moderate levels of severity, and 50% are likely to experience mild levels of severity. For rats 36% are likely to experience moderate levels of severity and 64% are likely to experience mild levels of severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We develop compounds that can be used for cancer therapy, cancer diagnosis, or both simultaneously.
It is essential to prove that these compounds are both safe and effective before we test them in humans, and only living beings can reproduce the complexity of human biology. In this project, we propose the use of rodents since they are a low order animal species with a significant resemblance to our biology.

Which non-animal alternatives did you consider for use in this project?

We systematically start any experiment by non-animal alternatives such as computer modelling for predictions or lab culture of human tissues and cells that have been obtained from tumour samples extracted from patients.

Why were they not suitable?

These non-animal alternatives can only replicate a very small fraction of the complexity of tumours inserted in a living being. For example, a substance that is effective at killing tumour cells in petri dishes may cause organ failure when injected in a living being and is thus not suitable for therapy.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the number of animals based statistical consideration and on the previous outputs of this group in the past. This is a 5-year project in which we propose to develop around 5 compounds for cancer

diagnosis and therapy, but the success rate of new compounds is small and thus we will need to develop and test a great number of compounds in order to obtain 5 that are successful. Testing new compounds is a complex process and their success or failure may not be evident until significant research has been done; thus any one molecule, whether successful or not, may entail the use of a large number of animals.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We are an imaging-based group which means we can acquire information through imaging rather than having to kill the animals every time we require it; this allows us to maximise the data generated by one animal, thus reducing the overall number of animals needed. Furthermore, we reduce animal use by reducing the number of controls needed and we do not use excess number of animals than required for relevant statistics.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Apart from the non-animal alternatives described, we will minimise the number of animals by having consistent experimental techniques thus reducing variability, by obtaining as much information as possible from one animal (for example, acquiring as many organ samples as possible at every cull).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice and rats are the most suitable models for this type of research because they share close resemblance to human biology and models of human cancer can be replicated in these animals. Mice will be used whenever possible, with rat models only used whenever unavoidable. In the majority of our work, we will use immune-deficient mice (mice that have been genetically engineered to have a weakened immune system) because they allow the growth of tumours without rejection, minimising the overall number of animals needed. Most tumours will be implanted subcutaneously (i.e. under the animal's skin) because these are valuable models of human cancer but cause little discomfort to the animal. More complex tumour models (such as tumours grown in their organ of origin or metastasis models) will be used only if the type of information needed cannot be obtained with subcutaneous models. We use imaging as a means to acquire data, which does not require the animal to be killed whenever information is required, thus reducing the number of animals needed. Furthermore, any procedures that cause moderate discomfort to the animal will be carried out under anaesthesia. Recovery from anesthesia will take place in a warm quiet environment.

Why can’t you use animals that are less sentient?

Less sentient animals are further apart from human biology. Furthermore, we consider that is ethically superior to use adult animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinement measures carried out in this project will include: use of anaesthesia and pain-relief medication whenever possible as well as enrichment of the animal's environment. We will frequently monitor animals, especially during treatment, and will increase monitoring if any adverse effect manifests; depending on the severity of harm, the animal will be treated or culled. Tumour monitoring will also be performed through imaging techniques. Extensive characterisation of any substance prior to injection in animals and having clear limits on animal injections will minimise the likelihood of adverse effects and suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the NCRI guidelines for the welfare and use of animals in cancer research, as well as LASA, PREPARE and ARRIVE guidelines together with body condition scoring guidelines as detailed in the protocols.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Frequent meetings are held at our institution on the advances in the 3Rs and their implementation. All staff working under this licence will attend these meetings and frequent advice regarding the well-being of animals will be sought from the vets and animal care staff.
We will follow external sources including National Centre for Replacement Refinement and Reduction of Animals in Research resources (website https://www.nc3rs.org.uk/) and NCRI guidelines.

Home Office

NON-TECHNICAL SUMMARY

88. Improving gene expression from viral vectors for gene therapy applications

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The overall aim of all work to be performed under this project is to develop improved gene therapies. In doing so we will develop, screen and evaluate novel tissue-specific and regulatable systems for testing in small rodent models.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

We plan to use new tools in animal models, mostly mice, as a means to demonstrate improved preclinical efficacy of gene therapy expression systems. These tools will be administered to wild type rodents to establish the bio-distribution and efficacy of gene expression. The project will further initiate a systematic pathway towards translation of the vectors tested in this project into clinic, whereby expression cassettes tested and validated here will be integrated into clinical programs ongoing at parental company. Our newly synthesized genetic tools are specifically designed to tackle particular diseases by targeting gene expression to those organs or tissues most affected in the disease. This will result in improvement/ enhancement of current available therapies, and the development of new treatments. As we are addressing many inherited/acquired diseases, where no treatment is currently available, our approach is of crucial importance to the field of gene therapy, enabling effective targeting of the diseased organ (or organs) and contributing potentially curative therapies to a wide variety of central nervous system, neuromuscular, cardiovascular, haematopoietic and metabolic genetic disorders.

What outputs do you think you will see at the end of this project?

The benefits will include:
Creation of novel tissue-specific or regulatable systems to control gene expression especially tailored for inherited/ acquired diseases. This is will greatly help with combating of many human difficult incurable diseases by creating safer and efficient therapies
Information from first round screening in small rodents to further improve on the development of gene expression systems that are translatable to the clinic.
Optimised gene expression systems to develop new clinical candidate gene therapy drugs targeting many neurological, neuromuscular, cardiovascular, metabolic, and haematopoietic disease that will be used to support new IND applications and go into patients
Development of new intellectual property and the dissemination of knowledge by means of publication and presentation in scientific conferences.

Who or what will benefit from these outputs, and how?

Our parent company is well positioned to exploit the results of these initial pre-clinical research studies. It is well-funded and has developed substantial resources in translational research, regulatory affairs and large-scale commercial manufacturing of viral vectors for gene therapy purposes. It has already produced vectors for a number of clinical trials, which have been managed both internally, or in collaboration with large biotech or pharma partners.

How will you look to maximise the outputs of this work?

We have a wide and excellent network of collaborators both in academic and industrial sectors and we are making a good use of this networking by sharing ideas, knowledge, and information. Our parent company has built numerous partnerships with other global pharmaceutical companies who will be interested in employing our technology in their clinical programmes to benefit patients eventually. The expected output of this project will be of crucial importance for the successful development of novel gene therapies targeting many incurable inherited/ acquired diseases.

Species and numbers of animals expected to be used

- Mice: 3500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In this project we will focus on the use of mice. As all mammals have almost the same regulation processes of gene expression, mice represent the most suitable animals to assess gene expression. They represent the lowest sentient mammalian species. Hence, we have chosen them because they have long been used to produce the well-characterised preclinical screening models of many diseases.

Typically, what will be done to an animal used in your project?

Animals will be used in this project for screening and validation of our novel gene expression systems, which are designed to especially target diseased organs of interest. We are not planning to use any severe surgical procedures, or invasive protocols. Instead we will be administering our tools via common and minimally invasive routes like IV, IM, IP and SC following local guidelines and then to be followed by non-invasive monitoring. Our focus will be on using adult mice at about 6-8 weeks for short term durations. At the end of each experiment, the mice will be sacrificed humanely by schedule-1 procedure or by perfusion under terminal anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

We are not expecting our administered reagents to cause any severe harmful effects on animals like weight loss, tumours, or abnormal behaviour. Perhaps animals will suffer from transient stress or mild pain after injections. We are expecting these effects to disappear very quickly and settle within hours without any need for intervention. However, effective pain relief and anaesthetics will be used carefully when there will be any need. Any animal will be immediately killed if it shows signs of suffering that is greater than minor and transient or in any way compromises normal behaviour, under guidance of the NVS. We will be looking for any general debilitation and persistent signs of distress or pain include reduced mobility, hunched posture, piloerection, rough ungroomed coat. If signs continue for 24 hours, we will seek NVS advice as to whether end point has been reached or whether symptomatic treatment can be carried out. If animals do not respond to treatment or show further deterioration, they will be killed by a schedule 1 procedure or by cardiac perfusion under terminal anaesthesia.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

No animals are expected to exceed mild discomfort.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this project is to screen and pre-clinically validate our novel gene expression systems, with the ultimate goal to apply our systems in gene therapy. To serve this purpose, we need to study gene expression that is controlled by our tools in mammalian systems, and very often, in different organs, tissues, and cell types simultaneously. As all mammals have almost the same regulation processes of gene expression, mice represent the most suitable animals to assess activity and functionality. We are not aware of any alternative method that allows evaluation of such responses other than using animals.
In this context, the FRAME website (www.frame¬uk.demon.co.uk) and NC3Rs website (www.nc3rs.org.uk) that lists alternatives to animal research have been examined without success. Furthermore, *In vitro* and *in silico* techniques are also not sufficiently advanced (and are not likely to be so for some considerable time) that they can model the integrated actions of the nervous system, for example. For these reason, *in vitro* studies using immortalised cells are of limited use and i*n vivo* models are the only way to fully evaluate the effects that a novel therapeutic agent will have. Importantly, the main purpose of this project is to conduct preclinical evaluation of novel therapies in relevant rodent models of disease. As part of this process towards clinical translation, medical regulatory bodies require *in vivo* studies in a relevant mammalian model as part of the traditional approval path to the clinical. However, wherever possible we always attempt to use *in vitro* models in this process to minimise any *in vivo* testing. An example of this is that, where possible, we test all therapeutic agents routinely and thoroughly on cell lines first in order to validate their quality, purity, efficiency, and lack of cytotoxicity before any further steps into *in vivo* models. These *in vitro* studies will be done before any *in vivo* studies. We continue to keep abreast of the latest developments and potential application of new technologies as potential replacements to certain aspects of *in vivo* work.
Therefore, we need to use animals in our studies because many systematic questions of gene regulation require better understanding that are not possible or feasible at present alternatively. We have always considered the use of animals ethically, but as we are not aware of an alternative way to evaluate gene expression *in vitro* that would enable this proposed study to be carried out successfully and in a practical manner, we believe their use to be justified in this instance .

Which non-animal alternatives did you consider for use in this project?

In silico computer modelling.
In vitro Cell line cultures.
*In vitr*o Organoid systems.

Why were they not suitable?

The diseases we aim to study are multisystem diseases, which cannot be modelled in vitro, neither in single cell cultures nor in mixed culture or organoid systems. Available non-animal based alternatives are of limited use and they cannot be fully representative of human disease models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals used will be chosen based on prior experience in experiments of this kind and with an aim to reducing this number but retaining effective statistical power in all cases. When performing mouse work, we calculate the minimum number of mice required to be sure that we will get an answer. We will also seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points. For all the experiments proposed we will use a group size which is the smallest compatible with achieving statistically meaningful and robust results.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We widely employ *in silico* designs and analysis in all our projects by using algorithmic, computer modelling and simulation. All our expression systems are initially tested, validated, and selected *in silico* efficiently before moving to apply them in our *in vitro* assessment ,where relevant, and eventually in *in vivo* studies. Likewise, we will also use bioluminescence-based biosensors to detect protein expression in live animals, rather than sacrificing groups of animals at repeated time points or, for example, performing repeated blood collections.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will make a good use of small-scale studies using minimum numbers of animals to get preliminary data before scaling up to bigger studies. Moreover, we will also endeavour to control experimental variables by making experimental material more homogeneous to reduce variability in the response. To achieve that we use viral material that has been subjected to rigorous QC and QA (these capabilities are located on-site), observe participant animals under similar biological/environmental conditions, employ standardised measurement methods, use calibrated equipment, perform all end-point analyses at the same time point where possible and blinding experiments to observers and testers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The principles of refinement will be adhered to throughout experiments. Effective pain relief and anaesthetics will be used rigorously. Also, we will be widely utilising biosensors which is a non-invasive approach for animal whole body *in vivo* imaging, providing a good refinement practice. Animals will only be used in our experiments if

they are in excellent health. Animals will be housed communally in IVC and in many cases in enriched environments to maximise social / welfare / rehabilitation considerations.

Why can't you use animals that are less sentient?

In vitro cell culture studies will be conducted, where possible, before animal experiments to select effective tools for gene manipulation. This reduces the numbers of animals required. We require a mammalian model as the studies need to correlate to the human organs' physiology and immune system, and mice represent the lowest sentient mammalian species. Furthermore, mice are the lowest species commonly used in experiments of this kind over the last 50 years meaning a great deal is known about their anatomy, neurophysiology, genetics and behaviour.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Generally, we will adopt good planning and scientific methodology throughout to avoid unnecessary suffering for animals by implementing:

1) Careful assessment of the initial and continued experimental design.

2) Expanding our background knowledge for relevant research, procedures, and findings.

3) Staged and gradual approach through small size studies.

4) Teamwork and resources, skills, staff performance the availability of suitable equipment and facilities.

5) Mice are monitored, by both staff and by our own team members. All our procedures will be done early in the day so that the animals have time to recover and have them under observations. Where possible, we will avoid conducting procedures on the last day of week.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidelines at National Center for the Replacement Refining & Reduction of Animal Research (NC3Rs).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

During the project we will continually review relevant published literature, comparing our objectives with those previously undertaken by others, and assess whether the results and conclusions of newly published studies have any impact on our experimental design. Importantly we will keep abreast of publications released by the National Centre for the Replacement Refining & Reduction of Animal Research (NC3Rs) for updates, guidelines and regulations.

Home Office

NON-TECHNICAL SUMMARY

89. In vivo studies of ADP-ribosylation signalling

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, embryo, aged
Zebra fish	embryo, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to gain a deeper understanding of the biological processes regulated by the modification ADP-ribosylation. ADP-ribosylation is a kind of chemical signal found in biology, responsible for various cellular processes - most of which are still largely unknown. We plan to do this by analysing novel GAA (genetically altered animals) (mice and zebra fish).

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Ultimately there are numerous **fundamental gaps** in our knowledge with regards to the normal biological role of ADP-ribosylation. Whilst we make advances other areas of ADP-ribosylation still almost nothing is known about the impact in a living being. ADP-ribosylation is a wide-reaching process with potentially significant impacts on behaviour/brain disorders, metabolism/diabetes/obesity and cancer/ageing which we will investigate here.

What outputs do you think you will see at the end of this project?

Major outputs:

1) Novel GAA (genetically altered animals) models (zebra fish and mice) to study ADP-ribosylation in a normal biological context.

2) Investigation of clinically relevant outcomes from such GAAs including novel aspects of ADPribosylation in neurobiology, metabolism and cancer.

3) Validation of treatment strategy for cancer

4) High-quality high impact publications.

5) Outreach to general public.

Who or what will benefit from these outputs, and how?

Research community (many fields but particularly the ADP-ribosylation field where such *in living being* knowledge is fundamentally missing) will benefit from the availability of novel GAA models and the new data generated by them. Both immediately as proof of concept – i.e. if they can target a protein for inhibition because the KO (knock out - deletion of gene in a living being) is survivable – and over time as further insights may inform their own research directions. Other researchers may start their own line of investigation with our GAA models as we would freely distribute them as requested.

Long term, the **general population** and **health care providers** will benefit from novel insights into three major areas of human disease;

Neurobiology – 10 million people are living with a neurological condition in the UK (globally the WHO puts this number in the billions). There are over 600 types of neurological conditions including stroke, migraines, autism, Parkinson's, Alzheimer's, dementia, motor neurone disease which has a significant cost to the NHS; £3.3 billion was spent in 2012-13 on neurological services. In addition, the burden of living with a neurological condition for sufferers and their carers is huge (14% of social care budget). Defects in ADP-ribosylation pathways have been directly linked to several neurodegenerative disorders as well as autism.

Metabolism – There are 3.8 million people living with diabetes in the UK. The cost of treating diabetes and its complications to the NHS is estimated at £14 billion a year (2018 data – 10% of the budget). Further, the

prevalence of diabetes is estimated to rise, especially considering that over 60% of the adult population are overweight or obese (increases the risk of diabetes up to 13-fold). Diabetes is a chronic, lifelong condition in which a person cannot regulate their blood sugar level. ADP-ribosylation genes have been implicated in metabolism including mutant mice where the blood sugar level is altered. Additionally, some ADP-ribosylation genes are expressed in the mitochondria a major site of energy and metabolic functions in cells.
Cancer – There are about 0.4 million new cases of cancer a year and around 130,000 cancer related deaths. The annual NHS costs for cancer services is huge, £5 billion, but the cost to society as whole is greater still at £18.3 billion (2015 data, including loss of productivity etc.). Some ADP-ribosylation genes have already been successfully targeted as cancer treatment (for example the drug olaparib a PARP1 inhibitor is now used to treat breast and cervical cancers) however given the wide involvement of other ADP-ribosylation genes in a variety of human cancers it is likely targeting these other genes could also be a valid strategy.
Total persons directly affected by one of these major disease areas in the UK is **14.2 million** at a total cost to the NHS of **22.3 billion** annually. This does not include those people at risk of disease (for example the 62% of obese adults in the UK) whom might benefit from preventative measures. Thus long term the **drug discovery/pharmaceutical industry** could benefit as well since our research will unlock new functions of the ADP-ribosylation pathways, including ways in which the ADP-ribosylation pathways might be targetable for disease prevention and treatment.

How will you look to maximise the outputs of this work?

Availability of new GAA models to the rest of the research community (through distribution to international mouse consortiums) thus widening the impact and number of fields our GAA models can be utilised in.
We readily collaborate with many scientists who are experts in their own field thus increasing the fidelity and impact of our science and its reach across several fields.
Research will be published in scientific journals and presented at conferences locally and worldwide. We keep an up to date website and will engage locally with Ley public through out-reach programmes, and other avenues.

Species and numbers of animals expected to be used

- Mice: 26,500
- Zebra fish: 6,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice and zebra fish all life stages.
Mice and zebra fish are physiologically and metabolically and genetically very similar to humans and they have relatively short generation times (baby-to-baby length 10 weeks for mice and 3 months for zebra fish) making them an ideal model of choice for generating genetically altered mutants. We can use the new mutants to study novel aspects of ADP-ribosylation in biomedical research such as behaviour, metabolism and cancer development.

Typically, what will be done to an animal used in your project?

Typical procedures performed during this project

~85% Breeding and maintenance. The vast majority of animals on this project will only be used on breeding

and maintenance protocols. Animals (mice and zebra fish) whenever possible will live in social groups with cage/tank enrichment in state of the art facilities until being killed by an appropriate humane method. Most mice are unlikely to suffer any project harms, not even mildly (called subthreshold to indicate no adverse effects whatsoever). Most zebra fish will be mild as they will need fin clipping as a means to genotype them (determine if they are mutant or not). Fin clipping involves taking a small section of fin, which does not hinder the fish and is performed under anaesthetic.
~5% Ageing and harmful mutants. Other animals will be aged and monitored for the development of mild-moderate conditions due to the presence of harmful mutations, such as neurodegeneration, metabolic defect and cancer.
~5% Behavioural testing where mice are subject to mild tests of naturally, appetite (mild food restriction) or aversely (bright light, loud noises, withdrawal reflex) motivated behaviour. A battery of tests lasts about 7 weeks (one test per week) and animals are usually tested again in old age.

~1% Metabolic testing where mice may have mild food restriction, administration of non-toxic substance (by injection or gavage) and limited blood sampling (4 times in 2 hours). A series of tests may be conducted over 4 weeks and animals can be retested again in old age.
~1% DNA damage testing where mice receive toxic agents (either irradiation or injected drugs) at short time points (<72 h) (before clinical symptoms present, mild). Or mid-term (50 days) follow up to a non-lethal dose (mild to moderate).
~1% Xenografts where mice receive an injection in their flank of human cancer cells to allow a tumour to develop. Mice may also receive treatment (typically new therapeutic agents).
~1% Models of carcinogenesis where mice receive toxic agents (either irradiation or injected drugs) over a short period to initiate cancer development. Depending on the method some mice also receive pro-inflammatory agents.

What are the expected impacts and/or adverse effects for the animals during your project?

Harmful mutants - such as neurodegeneration, metabolic defect and cancer. It is likely animals with such conditions feel generally unwell and experience pain, they may also have abnormal behaviour, difficulty moving, lose or gain weight. Animals will be closely monitored for harmful effects so that they are humanely killed at onset of pathological symptoms.
Aging - age-related welfare concerns include skin, eye and dental problems as well as increased risk of tumours and organ failure. These are mostly mild but long term conditions. Mice which appear generally unwell and/or have detectable tumours will be humanely killed.
Behavioural - acute stress from removal from home-cage (social animal, misses cage-mates) plus stressful stimuli such as bright lights/loud noises. May induce mild transient anxiety in mice.
Food restriction - mice should get hungry but not starving (mild and transient), just sufficient to motivate behavioural tests or get baseline blood chemistry.
Toxic/inflammatory agents - loss of appetite, weight-loss, diarrhoea, anaemia, likely pain and generally feeling unwell. These effects usually occur 7-14 days after receiving a dose and usually mice get better on their own but we do provide more palatable and easy to access food to ease suffering.

Xenografts - tumour development over a period of weeks (3-6). Mice with these subcutaneous tumours usually only have mild inconvenience since there is no internal tumour burden, but it is probably still uncomfortable to have a tumour on your flank.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice

80% Subthreshold

10% Mild
10% Moderate
Zebra fish

95% Mild
5% Moderate
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Currently no suitable alternatives exist for a number of important basic biological questions which we wish to study in this project, as follows;
Are our gene of interest (GOI) essential for and/or critically involved in;

- life, normal growth, longevity and fertility?
- behaviour, neurodegeneration and other neurological conditions?
- appropriate metabolic responses?
- DNA repair and cancer development?

Additionally, it is important to validate any therapeutic strategies in a living system to best assess their success.

Which non-animal alternatives did you consider for use in this project?

The vast majority of the work in our laboratory utilises alternative methods to study the processes regulated by ADP-ribosylation including: use of alternative species, cellular culture, biochemistry analysis, protein-shape analysis, evolution biology (how similar genes are between different species like from bacteria compared to human) and literature review (what is already known, what has already been done). With particular focus on replacement we implement two main methods;

1) Use of alternative species such as; bacteria and yeast and fruit flies (instead of zebra fish & mice) and;

2) Cellular culture (growing human or animal cells in dishes for experiments; such as cell growth and death).

Why were they not suitable?

These questions can only be answered using animals since no cell culture nor computer technology is currently sophisticated enough to replace the complexity of a whole living organism. Additionally, not all genes are conversed between flies and humans hence the use of mice or zebra fish is still required to study the effect of KO on these genes.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Mouse: Breeding and maintenance is calculated based on minimum requirements for safeguarding the line, replacement of breeders at 6 months, 2-3 breeding pairs and a few stock as back up in the case of infertility. To test fertility, expand breeding for cohort generation (for us and our collaborators) we would set up further crosses which is also included in this estimate. These numbers are multiplied by the number of strains we are expected to have. Experimental cohorts often require age/sex matched controls (within a week for metabolic testing within 3 weeks for behavioural testing) thus these are large breeding setups. We don't often use the heterozygous mice (half mutants) so these are killed as surplus. Experimental numbers are calculated based on average group size, expected number of groups, how many strains we expect to test and extra numbers in case we have to repeat.
Zebra fish: Breeding and maintenance is calculated based on minimum requirements to safeguard the line and for experimental numbers. We currently have only 1 zebrafish line and are not currently planning to expand but zebra fish produce a lot of offspring (up to 200 eggs at a time) hence the high number allowed under the licence. As with mice, we tend not to keep the half mutants (except for breeding) so they are killed as surplus. NOTE: this is a reduction from our previous licence.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

During planning phases of an experiment we communicate with other researchers in relevant fields and techniques to confirm our strategy and receive up to date guidance. Typically, this includes reexamination of the current literature, noting experimental setup and expected outputs and group size, then putting a draft plan together and contacting our collaborators for advice/input. In the event that we don't have access to a set of expertise we will contact the Home Office Liaison officers who can direct us to local support. When effect is unknown we will test a small amount of animals first (pilot study) to inform our design.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Efficient breeding strategies are a key component of managing our animal numbers. We require age matched (sometimes within a week) controls of adequate numbers for large experimental groups.
Breeding strategy for minimal colony management is also essential for keeping overall numbers low.
Another strategy is freezing mouse sperm (or fish eggs) to stop breeding animals and preserve the special genetic alteration indefinitely. We often routinely harvest animal tissues for archival purposes and to be able to go back to the precious biological material if a later need arises.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Animal models: Mice and zebra fish, all life stages
Methods:

Breeding and maintenance. The vast majority of animals on this project will only be used on breeding and maintenance protocols. Animals (mice and zebra fish) whenever possible will live in social groups with cage/tank enrichment in state-of-the-art facilities until being killed by an appropriate humane method. Most mice are unlikely to suffer any project harms, not even mildly (called subthresh-old to indicate no adverse effects whatsoever). Most zebra fish will be mild as they will need fin clipping as a means to genotype them (determine if they are mutant or not). Fin clipping in-volves taking a small section of fin, which does not hinder the fish and is performed under an-aesthetic.
Ageing and harmful mutants. Other animals will be aged and monitored for the development of mild moderate conditions due to the presence of harmful mutations, such as neurodegeneration, metabolic defect and cancer. Animals will be closely monitored for harmful effects so that they are humanely killed at onset of pathological symptoms.

Behavioural testing. Mice are subject to mild tests of naturally, appetite or aversely motivated behaviour. Not expected to cause more than transient unease/mild anxiety, in addition we perform testing in escalating order of severity, thus we may avoid the need for harsher tests altogether. We do not perform the highly aversive water immersion tests or inescapable foot shock tests.
Metabolic testing. For each test mice may have mild food restriction (minimum required time for scientific purposes), administration of a non-toxic substance (once) and limited blood sampling (over 2 hours only). This is the minimum sampling and administration of substance that still produce valid scientific data. Animals are allowed to recover a week between tests.
DNA damage testing. Mice receive toxic agents (either irradiation or injected drugs) at short time points (<72h) (before clinical symptoms present, mild). Or mid-term (50 days) follow up to a non-lethal dose (mild to moderate). We have a stringent welfare monitoring system in place to detect animals suffering from gastrointestinal syndrome that would not get better on their own. Mice suffering significant symptoms of gastrointestinal syndrome are humanely killed before end stage disease.
Xenograft studies. Immunocompromised mice receive an injection in their flank of human cancer cells to allow a tumour to develop. Mice may also receive treatment (typically new therapeutic agents). Immunocompromised mice are most refined since they will not have the severe welfare concerns associated with tissue rejection. Risk of infection is low since we house animals in state of the art, clean facilities. We will not allow the subcutaneous tumours to ulcerate or get too big to impinge on the animal welfare more than mildly. We are not using internal tumours in this protocol as they are harder to monitor and may have a higher welfare burden on the mice.
Models of carcinogenesis. Mice receive toxic agents (either irradiation or injected drugs) over a short period to initiate cancer development. Depending on the method some mice also receive proinflammatory agents. During the acute initiation phase of tumours, a proportion of mice will suffer symptoms of gastrointestinal syndrome (an unavoidable consequence of the toxic agents). These effects usually occur 7-14 days after receiving a dose and usually mice get better on their own but we do provide more palatable and easy to access food to ease suffering.

Why can't you use animals that are less sentient?

The methods described in this project require juvenile, adult and aged mice and zebra fish. They must me sentient for behavioural testing and they cannot be terminally anaesthetised for any of the tests. Ageing, behaviour, metabolism and carcinogenesis require whole organism participation over a period of time (days to years).
Use of a zebra fish model instead of mouse represents a refinement - where available and where we do not plan

to test more involved aspects of neurodegeneration, metabolism or carcinogenesis.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals are kept in social groups whenever possible, with cage enrichments and are regularly monitored for development of adverse phenotype. Animals are aged only for as long as clinical signs present to get maximal data with minimal distress.
We always perform testing in an escalating order of severity, thus we would only utilise harsher tests if there were clear biological evidence to justify it. Whilst this may mean we ultimately end up using more mice overall, in some cases it will also alleviate the need for the moderate tests in the protocols altogether. For example; currently we have only performed spontaneous behavioural testing
(considered the mildest) and will only look to escalate to more aversive tests if there are good biological reasons to do so.
The same is true with our DNA damage protocols; that is before performing an *in vivo* radiation sensitivity assay (where mice are kept up to 50 days following whole body irradiation without reconstituting them) we would first check if there was a disruption in the acute DNA damage response (where mice are killed prior to the onset of radiation sickness) > since the two are likely correlated.
When determining the cancer susceptibility of a new strain the chosen method will weigh up the harms to the scientific merit, thus if it is possible to perform the test without the use of toxic agents (perhaps by crossing to a cancer prone strain instead) we will always aim for that.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow the ARRIVE guidelines for publishing.
Welfare scoring for genotoxic agents: Endpoint Refinement for Total Body Irradiation of C57BL/6
Irradiation considerations: https://www.taconic.com/taconic-insights/oncology-immuno-oncology/rodentirradiation-considerations.html#footnote
PROTOCOL: An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumour progression
Tamoxifen Administration to Mice - Jonathan Whitfield, Trevor Littlewood, and Laura Soucek.
Comparison of Doxycycline Delivery Methods for Tet-Inducible Gene Expression in a Subcutaneous Xenograft Model -Christopher Cawthorne, Ric Swindell, Ian J. Stratford, Caroline Dive and Arkadiusz Welman.

Workman et al, guidelines for the welfare and use of animals in cancer research.
Routes of Administration : Shinya Shimizu
http://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/Routeadministration-4.pdf
LASA guidelines http://www.procedureswithcare.org.uk/lasa_administration.pdf

Table: Recommended maximum volumes and sites for dosing

ROUTE (Mouse)	Oral	Intra-peritoneal (I.P.)	Sub-cutaneous (S.C.)	Intra-venous (I.V.)	Intra-muscular (I.M.)
Common sites	Gavage, or in food or water	Body cavity	Scruff or flank	Tail vein	Anterior thigh
Maximum volume (ml)	0.4 (gavage)	1-2	0.5	0.2	0.05

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Attend 3Rs research day. Read the 3Rs newsletter and would contact them if we discovered any way to replace, reduce or refine animal welfare. Attend departmental welfare meetings. Member of international transgenic society to keep up to date there and readily collaborate with experts in other fields to ensure best practice and most refined method.

Home Office

NON-TECHNICAL SUMMARY

90. Infectivity and strain behaviour of prions

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

BSE, Scrapie, Zoonoses, Infectivity, Strain Typing

Animal types	Life stages
Mice	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To determine potential prion infectivity in various tissues that they may be risk assessed for consumption by animals or man or to validate prion disinfection methods.

The strain typing of prions to determine the strain properties of prion that are responsible for known and emerging animal Transmissible Spongiform Encephalopathies (TSEs) with the intention to identify animal TSEs that can behave like BSE with particular emphasis on the zoonotic potential and the ability of Bovine Spongiform Encephalopathy (BSE) to overcome species barriers.

A retrospective assessment of these aims will be due by 14 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

TSEs are chronic, incurable and fatal diseases of the brain caused by pathogens which mainly consist of a single protein termed prion. TSEs cause diseases in both humans and animals, including diseases that are zoonotic (passing from infected animal to man such) as BSE. All these diseases are currently untreatable and ultimately fatal.

This research is linked to statutory TSE surveillance schemes and it aims to minimise the impact of TSEs in animal and public health. The main focus of the research activities is to refine and enhance the existing bioassay methodology in order to improve the delivery of reliable data from sensitive surveillance cases as soon as possible.

What outputs do you think you will see at the end of this project?

The outputs and data that will be generated from this project will inform on the pathogenesis of TSEs, strain diversity of prions, qualitative and quantitative data regarding tissue infectivity, evaluation of decontamination methods and new in vitro diagnostic tests.

Who or what will benefit from these outputs, and how?

A large amount of this information is required by and has direct impact on government policy so it will be firstly communicated to the relevant authorities/laboratories as soon as data become available.

Data will be submitted to and peer-reviewed by funding bodies, peer review via preparation of manuscripts for publication and presentation at national and international meetings.

How will you look to maximise the outputs of this work?

As well as scientific publication, the research group is a member of various European and International collaborations, and advises policy makers in the UK and global organisations.

Species and numbers of animals expected to be used

- Mice: 9,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Transgenic mice are the biological system of choice for these studies. With the different prion transgenes inserted from cattle, sheep or bank voles the various transgenic lines reflect the susceptibility of theses host species. Compared to the host species they offer considerable advantages on speed (months rather than years) and physical size, social structure and husbandry requirements are conducive to humane care in containment laboratory settings.

The established model uses mice at a few weeks of age as they are fully immunologically developed and dependent on the type of experiment being undertaken may have to last up to 1000 days.

Typically, what will be done to an animal used in your project?

The mice used in the project are transgenic. The transgenes are not harmful and all breeding is done under this project, Protocol 1.

The mice used for experimentation will be given a general anaesthetic under which an identification microchip is inserted and a small amount of material that is likely to contain a TSE is injected intracerebrally. The mice will be recovered from the anaesthetic and then if the material contain TSE's some months later they will start to develop clinical signs of TSE. They are clinically assessed regularly by trained staff, once the clinical signs are confirmed the mouse is euthanised and the clinical and pathology information is combined for analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

There will be impacts around the intracerebral injection of TSE for which anaesthesia and pain relief will be given.

The other impact will be as they develop clinical signs of TSE. They are regularly clinically assessed by trained staff, once the clinical signs are confirmed the mouse is euthanised.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

35% mild (breeding only), 55% moderate 15% severe .

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 14 March 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Non-animal alternatives such as immunohistochemistry, Western blot, PMCA and RT-QuIC are used for statutory diagnosis and in research projects. However, none of these methods can assess infectivity or define fully strain characteristics. Only when information about infectivity or the finer detail of prion strain is required will the mouse bioassay be used.

Which non-animal alternatives did you consider for use in this project?

TSEs agents lack nucleic acids and do not induce immune responses. Therefore none of the immunological and genomic approaches available can be applied to detect them.

In pursuing our efforts to minimise animal use we are committed in trying new approaches in diagnosing TSE agents and characterizing their strains. Therefore we have undertaken research into emerging technologies in the TSE field including cell culture , organ culture and PMCA were tested to identify prions.

For the last 30 years there have been several attempts to use cell cultures for detection of infectivity or for strain typing. However, there has been incremental progress in this field confined mainly to mouse adapted experimentally produced prion strains. The organ culture methodology is more recent but it

has proved unreliable and similarly with the cell cultures it is not used any more in TSE diagnosis or research.

From these techniques only Protein Misfolding Cyclic Amplication (PMCA) promising in amplifying prions in vitro. However, this amplification may not necessarily be associated with infectivity. The organisation has contributed to this work under the previous licence and will further support the validation of this method as it represents the only realistic possibility in replacing partially mouse bioassays. Real Time Quaking Induced Conversion (RT-QuIC) is a more straightforward and reliable method for amplifying prions in vitro based on the same principle as PMCA. This technique is currently being established at the organisation included in the same project as bioassay and experiments have already been designed to compare it with mouse bioassays for TSE diagnosis.

Also in collaboration with other organisations we assess the ability of transgenic fruit fly (Drosophila Melanogaster) bioassays as an alternative to mammalian bioassays for detection of infectivity.

Under this Project Licence any emerging in vitro or non-mammalian bioassay method will always be validated against animal bioassays to identify if they can be adopted as suitable alternatives.

Why were they not suitable?

Currently only mouse bioassay can give the information about infectivity or the finer detail of prion strain as a clinical and pathological output is required to determine disease phenotype.

It is possible that in future PMCA, RT-QuIC or transgenic fruit flies may partially replace mammalian bioassays in TSE detection testing. However, currently no alternatives exist for the role of mammalian bioassay in strain typing.

A retrospective assessment of replacement will be due by 14 March 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The mice requested for this research represent a maximum estimate of the numbers required given current policy demands on established and emerging TSEs.

Based on data from the previous licences the number of animals under genetically altered mouse breeding protocol has been calculated as a realistic estimate to support production of mice for the experimental protocols.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Statistical input for studies has been and will continue to be sought to ensure validity of data from the organisations biostatisticians.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Research work during previous projects demonstrated that transgenic mice have superior sensitivity and specificity compared to wild type mice. As a result only transgenic mice are included in this licence. In infectivity experiments this results in a reduction from 20 down to 8 mice per group. When wild type mice were used for strain typing experiments serial passages were mandatory requiring at least 52 mice to characterise a strain. In contrast use of transgenic mice for similar experiments requires only 8 mice as strain typing can be achieved during first passage and serial passages are seldom required.

All transgenic lines are homozygous and their breeding in linked to experimental requirements thus avoiding any excess breeding.

A retrospective assessment of reduction will be due by 14 March 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project uses small panels of transgenic (genetically modified) mice for TSE strain typing. These mice have no animal welfare issues associated with the transgene. Anaesthesia and analgesia are used for the initial and only injection of potentially infectious material intracranially. Then there is an incubation period of generally several months during which time the mice are clinically normal but regularly neurologically monitored by trained staff. When consistent signs of TSE infection are seen the animal is humanely euthanised.

Why can't you use animals that are less sentient?

The evaluation of the pathology to define strains requires a fully developed central nervous system and clinical signs can only be observed in conscious fully developed animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We currently use post-operative analgesia, and monitoring regimes. The majority of inocula used are sterile for conventional pathogens, however in a small number of unavoidable cases there may be a concern over the inocula containing endotoxins. These inocula are heat treated and will initially only be given to a single mouse. If this mouse does not show any adverse reactions for 7 days the rest of the group will inoculated. If the mouse shows adverse effects it will euthanised. The inoculum then is diluted in 9 volumes of saline (1:10 final dilution) and the diluted inoculum is used to challenge a single mouse initially and if the mouse does not show any adverse reactions for 7 days the rest of the inoculations resume. If the mouse shows adverse effects it will euthanised and a further dilution (1:100) is tested as described above. If the single inoculated mouse shows any adverse effects in the 7 day post inoculation period then no further attempts to challenge mice with this inoculum are made.

When there is a concern over the presence of endotoxins increased post-operative monitoring regime is used to identify animals suffering from the effects and euthanase them if they are not going to recover from the effects.

For the diagnosis and identification of different types of TSEs at end of the experiment the mice have to have consistent clinical signs before humane euthanasia. Experience has shown taken to early, there are not sufficient pathological changes in the brain for analysis.

Regular monthly meetings involving the NVS, NACWO, animal care and laboratory staff , personal and project licence holders to ensure current knowledge is brought to bear. All aspects of the performance of the bioassay are discussed, what is going well and if there was anything that could be done better. If there are any suggestions for refining the procedure they will be considered and if appropriate, incorporated into the protocol.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Home Office The Harm–Benefit Analysis Process

Home Office Guidance to ASPA

Home Office Code of practice

RSPCA Guidance on Welfare of mice

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I have regular contact with the NIO, NACWO and NVS through various forums and use of the library function which can scan for relevant publications. In developing this work I have been in contact with other researchers outside of the organisation who also specialise in the TSE field and any viable alternatives to mouse bioassay will be continually pursued.

A retrospective assessment of refinement will be due by 14 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

91. Injury and repair of the Spinal Cord

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

spinal cord injury, axon repair, gene therapy, central nervous system, regeneration

Animal types	Life stages
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to promote regeneration of the neurons of the Central Nervous System after Spinal Cord Injury.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This work is important because spinal cord injury (SCI) is a devastating event, often resulting in permanent dysfunction of the Central Nervous System (CNS - consisting of the brain and the spinal cord). Treatments to restore function must enable the regeneration of the neurons that were damaged during the injury. The injury of mature neurons of the CNS results in a loss of function and sensation. This happens because the injured neurons cannot transmit information anymore. SCI severely limits an individual's ability to complete basic tasks, such as standing, walking, feeling pain or sensations.

These effects drastically impair one's freedom, safety and quality of life. Every year in the UK, over 1,000 people sustain a SCI in addition to the existing 50,000 cases of people living with paralysis. It is estimated that SCI costs the healthcare system £1 billion annually.

Unfortunately, to this day, SCI still remains a debilitating condition for which there is no cure. As of yet, no treatments that would promote the repair of neurons has completed the clinical trials and with damage being permanent (i.e., paralysis), patients are still waiting for an effective treatment to recover some mobility and sensation. A treatment promoting spinal cord regeneration would have a great impact on improving the quality of life of these patients.

Through our work in the laboratory using cellular models of regeneration (e.g., nerve cells in a culture in an artificial medium), we have identified several new ways to stimulate neuronal regeneration after an injury. We now wish to translate these findings to animal models of SCI whereby the complexity of the mammalian spinal cord can be better understood, and behavioural studies conducted. This licence will enable the testing of these new regenerative methods in the rat spinal cord with the hope that our novel technologies and discoveries will successfully progress to clinical trials.

What outputs do you think you will see at the end of this project?

Definitive spinal injury and recovery experiments should identify one or more new treatments that have the potential to improve recovery after spinal cord injury in human patients (i.e., start of clinical trials). Among these outputs, we have three main axes for this research project (in animals):

-At the end of this project, we will better understand how some molecules involved in neuronal growth are involved in the axon regeneration process. The axon is the part of a neuron which is damaged during injury and through which the information is "transported" to other neurons.

-We will also know if a molecule that we have identified as a new target can regenerate the spinal cord in the same way as it has been shown to regenerate the optic nerve after an injury.

-Additionally, new data will be collected and interpreted about neurons, glial cells and astrocytes (two different types of cells, present in the CNS, which are essential to the functioning of the neuronal networks).

This work will improve our understanding of the global picture following an injury.

It will help us to assess which are the right doses of our viral vectors to be used and the duration of the treatment (i.e., how long these vectors need to be expressed in the body). A viral vector is a harmless modified viral structure used as a precise and specific delivery system. Whilst being totally safe for the host, it allows drugs to reach a specific target/organ.

We will obtain this information by studying how long our therapeutic treatments will be present in the body and assessing the level of recovery.

Results arising from our use of viral vectors and other drug treatments will be shared with the research

community. The viral vectors that will be deemed the most effective should ultimately reach human patients via clinical trials.

Who or what will benefit from these outputs, and how?

The outputs of this work will be disseminated to academic scientists during the licence, whenever we have opportunities to share it at conferences or workshops, in order to avoid duplication of the work. It will also give us the opportunity to build new collaborations to improve the quality of our research for the duration of the licence. Moreover, we will publish our findings in peer-reviewed journals, allowing both academic and pharmaceutical scientists to benefit from our research and help us develop tools to treat spinal cord injury. Tools, particularly viral vectors (therapeutic tools using a harmless viral structure), will be made available to other researchers.
In the long term, we hope that our work will benefit patients and we hope to complete the preclinical work to begin a clinical trial.

How will you look to maximise the outputs of this work?

We will share the information found in our studies as much as we can. In addition to weekly lab meetings, we communicate regularly during meetings organised for the strong research network of groups in the field of spinal cord injury. These collaborations allow us to share workloads and ideas (depending on the expertise of each group), avoiding duplication of the experiments and accelerating the progress in this field.
We will regularly take part in national and international conferences and present work annually to establish new collaborations in order to share our knowledge. These collaborations also help us to optimize our protocols (i.e., techniques used during experiments and the design of the experiments).
We will publish regularly, both positive and negative results, to increase awareness and inform the community as to which treatments are worth progressing and which we feel would not be worth further experimental exploration. Our university is a leader in research and our work is taken seriously and considered respectable in other institutes. We will ensure that the published results are open access to maximise their impact and increase awareness of scientists from around the world and the public. **Species and numbers of animals expected to be used**

- Rats: 1260

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Spinal cord injury in humans leads to lifelong paralysis and loss of sensation. The aim of the work is to develop new treatments for spinal cord injury. Rats and humans demonstrate a similar pattern of recovery after a spinal cord injury. Rats are therefore a suitable species to model regenerative therapies. Non-mammalian animals do not show the same biology: invertebrates (e.g., insects) don't have a spinal cord, fish and most amphibia present a much greater recovery and regeneration after spinal cord injury compared to humans and utilise different mechanisms of repair. A mammalian model is therefore necessary.

Recovery from Central Nervous System (CNS) damage is typically greater in younger mammals. However, as most of the patients are injured during adulthood, the challenge in spinal cord repair lies mainly on modelling the repair of mature neurons of the CNS. Therefore, reproducing this type of injury requires adult animals.
Rodent spinal cord injury models are also highly developed with a large cohort of supporting literature and methodology. Rats also appear to be the best choice for the following reasons:
-Considering the anatomy and the size of the animal, it is much easier to produce a reproducible partial spinal crush in a rat than a mouse.
-The behavioural tests we use have been highly developed and refined. They provide robust and reproducibly valid scientific data which enables us to gauge the efficacy of our treatments.
-We have access to a large choice of different strains of rats, among which some are more suitable for behavioural studies. These strains acclimatise well to our behavioural tests which are often enjoyable for them.

Typically, what will be done to an animal used in your project?

Rats are required for all the protocols defined below and will undergo only one of them. Each protocol is a combination of different procedures. The procedures used in this project are either surgical (i.e., the rats will undergo a general anaesthesia followed by recovery) or behavioural (i.e., different abilities or sensitivities will be tested). In total, the rats will undergo 2 general anaesthesia (insensitivity to pain, especially as artificially induced) with surgical procedure(s) and followed by recovery, at the maximum.
Here are the different **procedures** our rats can undergo:
Injections *(protocols 1, 2 and 3)*: Rats will receive an injection of a new potential treatment, into the nervous system (in the brain, spinal cord, dorsal root ganglia (DRG) or sciatic nerve), which should promote neuronal repair after a spinal cord injury (SCI), or a fluorescent tracer (control viral vector or chemical tracer).

The viral vectors (active or control) and the fluorescent tracer are injected with a needle, under general anaesthesia, in one or several sites (to extend their diffusion and expression). A viral vector is a harmless modified viral structure used as a precise and specific delivery system. Whilst being totally safe for the host, it allows drugs to reach a specific target/organ. The rats recover well after the injections.
The injection of a fluorescent tracer allows us to visualise some structures from the central nervous system. This is essential to be able to analyse the effect of our treatments, once we have collected the organs after the rats have been killed.
Spinal cord injury (*protocols 2 and 3)*: Some rats will undergo partial SCI that results in partial disability for approximately one week. A partial disability means the rats can show a small motor impairment, but will still be able to move around their cage and to eat and drink normally. Additionally, we expect the rats to have issues completing meticulous tasks such as grabbing a pellet of food, that require fine skills for the use of their paws. The partial injury of the spinal cord used in our research represents the most common type of SCI encountered by patients.
Behavioural testing *(protocols 2 and 3)*: Some rats will undergo various behavioural tests to assess their functional recovery after an injury. Here are some examples of tests:
-We can record their gait on a glass runaway to assess any impairments.
-We can record their ability to grab a pellet of food through a grid to assess fine motor skills.
-We can test their sensitivity to cold by applying ice on a paw and timing the withdrawal.
These behavioural tests are harmless for the rats and they actually often seem to 'enjoy' them.
Therefore, by combining some of the procedures above, we have been able to define 3 different protocols our rats could undergo.
Protocols

Protocol 1) The main objective of this protocol is to test how well our new therapeutic tools (i.e., viral vectors) are expressed in the CNS structures after an injection.

There will be an injection of a viral vector and/or a tracer into the CNS or into a nerve such as the sciatic nerve, which runs from the back into the leg. In our case, we used viral vectors as treatments because they will allow

us to modify the level of expression of some molecules into the nerve cells, which are involved into the repair process of damaged nerve cells.
Injection of a viral vector in this protocol will allow us to validate its use in a rat model: we need to validate that this treatment is expressed in the brain and the spinal cord before assessing its efficiency in association with an injury. These viral vectors express, most of the time, a fluorescent molecule which allows us to visualise the structures of the brain and spinal cord which the treatment has reached. If the viral vector is not fluorescent, we will inject a separate tracer to visualise the structures. Then, we will be able to visualise the expression of the treatment with an extra protocol, after the death of the rat.
This protocol will also allow us to determine the best route and dose of administration for our viral vectors.
This protocol lasts between 2 weeks and 3 months in general.
Protocol 2) The main objective of this protocol is to study the recovery of a rat, after a spinal cord injury without repair.

This protocol will allow us to study the morphology of a spinal cord injury (how the injury modifies the anatomy and the expression of some molecules into the CNS) and to assess the recovery of the rats, after the injury, without a treatment.

An injection of a tracer may be used to help to visualise the modifications of the structures of the CNS may happen before, during or after the spinal cord injury, depending on the tracer used.
To study and assess the level of spontaneous functional recovery after injury, some rats will undergo under behavioural testing to assess their movement, their agility or their sensitivity to various stimulations (e.g., heat, grabbing an object).
The behavioural testing may happen every day, with 3 different tasks maximum, and up to 12 weeks, after the injury.
This protocol lasts between 1 and 4 months.
Protocol 3) The main objective of this protocol is to study the recovery of a rat after a spinal cord injury with repair (i.e., administration of a viral vector).

The rats will have a spinal cord injury and will have an injection of a viral vector (or its control) to help to promote repair of the injured nerve cells. Depending on the viral vector, the injection will happen before, at the same time or after the spinal cord injury.
If needed, an injection of a tracer to help to visualise the structures of the CNS may happen before, during or after the spinal cord injury, depending on the tracer used.
To study and assess the efficacy of the treatments to improve the level of functional recovery after a spinal cord injury, some rats will undergo under behavioural testing to assess their movement, their agility or their sensitivity to various stimulations (e.g., heat, grabbing an object).
The behavioural testing may happen everyday, with 3 different tasks maximum, and up to 20 weeks, after the injury.
This protocol lasts from 2 to 6 months.
After the death of the rats, we will study the anatomy of the tissues from the central nervous system (brain and spinal cord) following the injury and the repair (if there was some) to observe the structure and see if we have managed to promote the repair of damaged neurons.

What are the expected impacts and/or adverse effects for the animals during your project?

The adverse effects which may appear during this project are related to surgical procedures and not from any behavioural test. They are as described below:
-Injections of tracers and/or therapeutic treatments (in areas other than the spinal cord or dorsal root ganglia (DRG) - DRG are nervous centres located just next to the spinal cord) may cause occasional pain, distress or discomfort. These adverse effects should resolve quickly after surgery (<72h) and painkillers will be provided

daily, if necessary and in consultation with the animal unit staff, as rats are monitored daily after a surgery.
-Injection of tracers and/or viral vector into the spinal cord and DRG may also cause occasional pain, distress or discomfort. These adverse effects usually resolve within 72 hours but may last up to 5 days post-surgery. The longer recovery is necessary due to cutting deeper into the tissue to access and expose the spinal cord and DRGs. Pain-killers will be provided daily if necessary (depending on the daily pain assessment), and in consultation with the animal unit staff.
Partial spinal injury leads to reduced mobility and a temporary disability (i.e., the general quality of life of the rats is not impacted) for around a week. Impaired movement can include dragging of legs and reduced agility. During this time the rats will be provided with access to ground level food and drink. We will also increase daily observations. Mobility usually improves within one week and monitoring will remain until rats are sufficiently recovered. Their recovery is assessed with a specific score sheet and indication sheet adapted to this surgery (e.g., clinical signs, assessment of gait) to help the animal unit staff. To aid recovery, a heated pad shall be attached to the underside of the cage (up to 48 hours post-surgery). Pain-killers will be provided daily and up to 7 days if necessary, and in consultation with the animal unit staff.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All rats will be in the moderate severity, which means they will undergo a general anaesthesia with a surgical intervention. Their recovery will be monitored and pain-free as pain-killers will be given to them regularly. And their quality of life is not impacted by any of the protocols we will use.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our group has been working on spinal cord injury for a long time now. Through these years of work, we have developed a strong understanding of models of regeneration, of nerve cells from rat, in culture: these nerve cells are put in small plastic dishes at 37˚C, with a special liquid, which allows us to keep them for several weeks. We chose this kind of cells, as they are the ones involved into the control of movement in animals and human and are actually damaged on the occasion of a spinal cord injury. Indeed, it is the part of the nerve cells through which the information for movement circulates through the spinal cord. Hence, when the spinal cord is injured, these nerve cells are injured and cannot transmit information anymore. This leads to partial -at least- paralysis. This model helped us to validate the different therapeutic treatments to promote the regeneration of these nerve cells, in the central nervous system, after an injury. However, in order to validate these new therapeutic treatments, we need to develop and use an animal model. This will allow us to test whether these potential treatments are working in a physiological environment and injury context. The context of the injury is very different in an animal compared to a plastic dish. In an animal, there are many physiological responses (inflammation, formation of scars etc.) which can happen and so impact the efficiency of the treatment. The injury also affects many nerve cells at the same time, not just a single one in a dish. To progress our treatments to clinical trials, we need to validate these studies through the relevant animal models.

Which non-animal alternatives did you consider for use in this project?

The laboratory has developed a new model of culture of nerve cells in plastic dishes to test the tolerance and the efficiency of our treatments before testing them *in vivo* (i.e., in an animal). This model of nerve cells predicts more accurately than other models of cell culture in plastic dishes which treatments will be effective at enabling the regeneration *in vivo*.

Why were they not suitable?

Nerve cells grown in culture dishes are useful for studying some aspects of injury, but they cannot replicate changes relating to a spinal cord injury, such as inflammation and scarring. Nerve cells grown in culture also behave differently to those found in a living organisation, including limited electrical circuitry and communication with other cell types.
It is therefore necessary to use animal models to assess the biological and behavioural responses, both following an injury and after therapeutic treatment. There is also a requirement to demonstrate that a treatment is safe and effective in animal models before progressing to clinical trials.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers were estimated based on statistical calculations of the number of animals needed in experimental groups to produce valid results and based on previous experimental experience. As the experimental methods are improved and refined, the difference between the results in the same experimental group decreases, and this will make it possible to use fewer animals with fewer complications during the experiments requiring a surgical step.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experiments are constantly assessed at the pilot stage (i.e., the first experiment conducted with a reduced number of animals to adjust some parameters before running the full experiment) and full experiment stages. This ensures that the correct number of animals necessary to achieve robust statistical results is used. The *National Centre for the Replacement Refinement & Reduction of Animals in Research* (NC3Rs) experimental design assistant is a tool which we use to help to design each type of experiment. It is a tool which we use regularly, particularly when there is new information available which would enable us to further refine our experiments.
The animals are placed in the experimental groups randomly: we do not decide beforehand which animals will be allocated to each group and the groups are homogenous.

We are performing ‘blind studies’ for the injections of our vectors: it means that the surgeon performing the injection does not know what is injected and is only told after the results have been analysed. Thereby, there is no bias in the analysis of the results.
During the behavioural assessments and histological studies (i.e., the nature and composition of animal tissues) that allows us to assess how effective our treatments are, researchers do not know which treatments have been used in each animal. This ensures that our researchers interpret all results without risking bias introduction.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Experiments will be clearly and properly planned, defining the objective and outcomes of each of them, before being performed. Methods will be discussed with the animal unit staff to be sure that all the necessary equipment is in place in order to be able to do the work in optimal conditions.
We will always perform pilot studies before undertaking a full experiment, to ensure that larger studies are as accurate as possible, and to avoid replication. These pilot studies allow us to assess if the experiment is well designed and to identify problems as well as formulate improvements early on in the licence. These pilot studies will also help us to check if we have the necessary staff and expertise to run the full study and that the equipment to process samples is working optimally.
When we perform tests on rats like behavioural ones, we multiply their frequency throughout the life of the rats, to have more robust results.
We will collect as many tissues as we can from any rat from our experiments to maximise their use: we systematically collect tissues to perform further studies (e.g. looking at the morphology of the tissues).
We are also coordinating with other groups to share animal tissues in order to reduce overall animal numbers.
We will plan our experiments in accordance with the guidance provided in the *Planning Research and Experimental Procedures on Animals: Recommendations for Excellence* (PREPARE) guidelines. This will not only mean we use the right number of animals but will also ensure our results are robust and reproducible.

We will follow the *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) guidelines when preparing our data for publication. In doing so, we will ensure our findings are complete and clearly presented when published, and are easily accessible to other groups. This should reduce unnecessary duplication of animal experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In this kind of experiment, the most appropriate animal model appears to be a quadruped as we need to assess the motor and sensitive functions, following injury and repair.
The rat is a better species to use than the mouse because it is easier to perform a reproducible injury on them due to their larger size; moreover, they are more responsive to behavioural tests. The performance during the behavioural tests can be further improved by selecting the most appropriate rat strain.

The animals will receive a partial spinal cord injury. The lesion (i.e. the injury of the spinal cord) model and assessment has been refined over the years so that only a partial injury is made, which produces a partial disability for a few days: animals will show a reduced mobility and agility. This impairment of mobility and agility does not affect their ability to eat and drink as usual. After a few days, animals behave normally in their cages. The behavioural tests are very sensitive and are used to detect minor disabilities (detectable with cautious study) and assess the recovery in animals after injury.

Why can't you use animals that are less sentient?

We cannot use invertebrates (e.g., worms), fish or amphibia, as they are not suitable to develop a treatment for human spinal cord injury. Some preliminary work on regeneration biology is done in nonmammalian species, but for experiments on recovery from spinal cord injury in humans and veterinary animals only mammalian species provide an adequate model: as we explained it, this animal model is necessary due to the anatomy of the spinal cord and the need for similarity with the human one.
We also cannot use animals that have been terminally anaesthetised as we need to assess the efficiency to promote nerve cell regeneration. Thereby, we need the animals to remain alive for several months after surgery. We need animals with nerve cells which have reached a mature stage to be representative of human injury: therefore, we need to use adult animals and cannot use embryos or immature life stages for our experiments.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have put in place a post-operative assessment sheet, specific to our model of injury or injection into the spinal cord area, to help the technicians to monitor better the recovery of the animals after surgery.
This post-operative assessment sheet will be refined during our work, depending on our observations and in collaboration with the animal unit staff. Likelihood of pain during the post-surgical recovery period will be controlled by the administration of pain killers, as directed by a veterinarian.
The behavioural tests used are not painful or stressful and are usually enjoyable for rats. In order to reduce stress, the assessor spends time with the animals to achieve familiarity.
Full training will be provided to new technicians who are unfamiliar with these procedures: we have filmed previous study procedures to show how we expect our animals to recover. These movies are available to help them to familiarise with the way our rats recover from a spinal cord surgery. It helps new technicians to learn how to assess our animals correctly and in turn, means that animals recovering in our experiments receive the same high quality and consistent level of monitoring and care they need.
We have also developed, if needed, the use of jackets to protect the surgical wounds on the back of the rats following a spinal cord surgery. Animals are acclimatised to the jackets a few days before the surgery and can wear them for a few days after the surgery until the wounds are healed. This prevents damage to surgical wounds, for example from back scratching.
It happens that some animals have to be single-housed following a surgery to prevent further damage to their surgical wounds (e.g. if wounds have been re-opened after playing with cage mates). In this case, whenever possible, we will divide the cage in 2 with clear plexiglass during the scarring of the surgical site. With this system, rats can see each other, which reduces the stress caused by the single housing.
Pain is not necessary for any of the studies and we will administer pain-killers to our animals when needed, as directed by a veterinarian. Regular discussion with a veterinarian will allow us to improve the management of pain, if any new and more suitable recommendation appears during the work.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Any new practices on animal work utilised by the establishment laboratory will be incorporated into our research. Excellent information is available on our establishment website, which is constantly updated with new 3Rs information.
The *National Centre for the 3Rs* (NC3Rs) website will also be regularly consulted to be sure that we are applying the latest recommendations in terms of refinement.
We are already following recommendations about the use of *vitro* models to reduce the number of animals as explained here: https://www.nc3rs.org.uk/vitro-model-spinal-cord-injury-replace-use-rodents
The *Laboratory Animal Science Association* (LASA) website provides updated information, especially regarding good research practice to perform aseptic (i.e., germ free environment) surgeries. (https://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf)
We will also consider any new publications in a peer-reviewed journal which is relevant to our field and which will offer new refinements for our protocols. (e.g. https://www.researchgate.net/publication/339667378_Refining_rodent_models_of_spinal_cord_injury)
To finish, we will also review regularly the best practice guidelines on substance administration volumes and frequencies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our establishment offers continuous training and advice through the training facility and the animal facilities. We will keep informed by regularly consulting the website they provide to be sure that we are not missing any new updates.
The *National Centre for the 3Rs* (NC3Rs) will also be the main reference to assess whether our experiments are matching the highest standards of 3Rs, and we will adapt our protocols if the recommendations evolve throughout the duration of this project. If any alternative to animal use materialises during this project, we will assess its suitability for our research.
Regular consultations on the latest practical guidance from *Laboratory Animal Science Association* (LASA) and the *Royal Society for the Prevention of Cruelty to Animals* (RSPCA) will provide other sources of new recommendations and advances.
We will keep our training up to date and will provide extensive training to any new lab member who joins the group to work on the project.
As a licence holder, I will stay updated by consulting information for licence-holders provided by our establishment and by speaking to other project licence holders.

Home Office

NON-TECHNICAL SUMMARY

92. Intestinal failure in neonates and adults: mechanisms, protection and repair

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Ischemia, Reperfusion, Nectrotizing, Colitis, Stem Cell

Animal types	Life stages
Mice	adult, neonate
Rats	adult, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The overall aim of project is to further our understanding of the pathophysiology of intestinal failure and test the efficacy of novel stem cell derived therapeutic strategies that may have implications for the treatment of these conditions in humans.
A retrospective assessment of these aims will be due by 16 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Lack of blood flow induces damage to the gut during neonatal as well as adult period of life, which results in severe consequences to human health. Presently, there are no treatments to cure patients who experiences such episodes. Previously, studies have shown that molecules produced by stem cells promote tissue regeneration when these molecules are given to an individual from whom the stem cells were initially isolated. Here we will test for the first time whether stem cells or molecules that they produce derived from one animal or human can alter the disease progression of a gut of another animal that has experienced periods of ischemia.

What outputs do you think you will see at the end of this project?

Knowledge gaps need be filled regarding the mechanisms of intestinal failure in two settings: Ischemia reperfusion injury (IRI) and Necrotizing enterocolitis (NEC) .

- Improved scientific understanding of the mechanisms of injury by NEC and its sequelae (short bowel syndrome and neurodevelopmental complications) is essential for the development of effective therapies against the disease. This will be achieved through analysing the tissue at the histological level, profiling the expression of key proteins that control the major mechanisms underpinning tissue homeostasis, as well as key gene markers reporting on tissue health.

- A short-term benefit of this proposal will be to define the mechanisms of pathology and how they can be modulated by stem cell factors in rodent models

- The key outputs will be the generation of new information, which will be communicated via publications in peer reviewed international journals.

Provision of this information will allow usage by third parties.
The outcomes of these studies will be made available to interested parties through a number of routes. Firstly, we will publish our work in international peer reviewed journals. Our work will also be communicated at national and internal conferences and workshops.
All raw data will be made available upon request following clearance for the provision of this information from the University Data protection unit.
The long-term benefits of this project will be the translation of knowledge gained in establishing the stem cell secretome based therapy to these pathologies. This long-term goal will require a tiered process that involves safety testing, testing in a non-rodent model for efficacy before entering a phase one clinical trial (first in human).

Our collaborators already have an established pig model for both conditions which will accelerate the translation of positive results from this licence should they arise. Furthermore, the translational route will be accelerated by partnering with clinical teams who are closely involved in the training aspects of this project.

Who or what will benefit from these outputs, and how?

The short-term benefits of this proposal will be gaining mechanistic insight into the two indications at multiple levels, ranging from whole organ function down to the identification of molecules that are perturbed as a conscience of the diseases. The identification of aberrant molecules is likely to be of particular benefit to third parties as they themselves could become therapeutic targets. Therefore, the short-term benefits are like to be important to not only the general scientific community but also to groups around the world working on these diseases.
Long-term benefits include the development of novel efficacious therapeutic strategies for patients with NEC and IRI for their improvement of their quality of life. This is an important target as NEC and IRI is associated with significant morbidity (short- and long-term) and mortality. Caring for such patients is difficult for relatives and healthcare professionals and has significant impact on limited resources. In order to achieve this, it is essential to appreciate that NEC and IRI are multi-factorial diseases. In the present project, we account for this by using two distinct models of “NEC-like” injury. The therapies we will investigate include stem cells or factors derived from stem cells in both a rat and mouse model of IRI and in rat for NEC by various routes of infusion relevant to clinical translation in animals at appropriate stages of development relative to the indication.
The clinical use of differentiated or undifferentiated stem cell conditioned media provides an attractive, less invasive, safer alternative (minimising cancer, reducing immunological risks) to stem cell transplantation. The benefits could be in future be reaped by those who suffer with ischemic-induced damage of the gut.
However, this is only the first step towards use in the community. Future studies will require safety testing a process that is likely to take two to three years after the successful completion of this project.
How will you look to maximise the outputs of this work?

We will communicate the results arising from this study by presenting our progress at both national and international conferences and meetings as well as communicating completed studies in the form of publications.

Species and numbers of animals expected to be used

- Mice: 1000
- Rats: 2300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rodents are being used in this project as they can be used to develop the symptoms of the diseases that are the focus of this project: Ischemic Reperfusion injury (IRI) and Necrotising Enterocolitis (NEC).
The IRI work and work on NEC will be conducted on adult rodents and new-born rodents respectively, as these developmental ages mimic the times of respective disease onset in humans. Both animal models were first developed in rat due to the size of organs that need to be manipulated. We will establish the protocols in the rat and then move to the mouse for IRI. The mouse is a more attractive model due to logistical reasons in the first

instance as they are more readily maintained (smaller cages, and cheaper to maintain) Secondly after demonstrating that we can perform IRI in mice we hope (through amendments to the license) to work on transgenics.. If unsuccessful, then we will conduct all our studies in the rat.
All work on NEC will be carried out in rat due to the size of newborn pups and the type of intervention that is required to induce NEC. This work in the first instance would be technically challenging, although not impossible in mice.

Typically, what will be done to an animal used in your project?

Typically, rodents will be maintained until the desired age and then be induced to develop a disease state, which will be achieved either through surgical intervention (Ischemic Reperfusion injury (IRI)) or treatment comprising of exposure to reagents and stressful conditions (Necrotising Enterocolitis (NEC)).
Thereafter they will be treated with a stem cell secretome, which has potential therapeutic properties.
The treatment will be allowed to have time to work, during which time the rodents will be monitored to assess the impact of the treatment.
The experiment will be terminated at a set period. The animals will then undergo physiological examination which will allow us to gauge how well the tissues are working. Thereafter the animals will be killed, and tissues collected for further investigation aimed at revealing how the treatment has altered the course of the disease.

What are the expected impacts and/or adverse effects for the animals during your project?

The interventions described in this license will compromise the working of the gut, which will have impact on both body weight and potentially other physiological processes. Examples of adverse effects could include hunched posture, piloerection, anorexia, septicaemia, abdominal pain, inactivity, foam coming out of the mouth.

Additionally, rodents may show signs of general malaise and decreased activity.
We will implement rigorous monitoring regimes which will allow us to identify any signs of adverse effects and depending on the nature of the adverse effect we will either stop the intervention followed by a recovery protocol or humane kill the animal to prevent further pain, suffering and distress.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The most severe category is severe which will be experience by no more than 15% of the animals in this proposal. A small proportion of animals will be used in studies to evaluate whether the treatments improve survival.
However through the use of a rigorous monitoring regime informing of when an animal shows signs of no longer being recoverable, we will perform humane killing to minimise pain, suffering and distress.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 16 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The objective of the project is to explore the clinical benefit of stem cell secretory factors on ischemic gut disease in adult and neonates. Mice and rats are the lowest sentient species that can be used to reach our objectives. Alternative experimental models for research into gut diseases have been developed including those carried out in test tubes or with whole animals including amphibians and fish. However, none of the latter faithfully replicate the condition in humans.

Which non-animal alternatives did you consider for use in this project?

With some aspects of the project, experimental animals can be replaced by in-vitro testing, and as outlined in the programme of work such in-vitro testing will replace intact animal experimentation wherever possible in the project. Such in-vitro testing will include appropriate dissociated cell culture, cell line testing and testing on organoid culture preparations appropriate for each disease model. Organoids technology is relatively new and allows scientists to grow a 3D collection of cells that have all the major cell types found in the human gut. Furthermore these have been used to develop models of necrotizing enterocolitis. We have extensive expertise in the development of gut organoids starting with tissues harvested from rodents.

Why were they not suitable?

While in-vitro studies are very informative on the impact of a disease or condition on a particular tissue, they have very little value when the nature of the disease or condition acts in the whole body. While organoids are incredibly informative when addressing key developmental issues related to the gut, they are presently still not a good model for studying the types of diseases investigated in this study. Organoid 3D architecture is not as regular as seen in vivo. More importantly organoids lack several essential components of the living digestive tract, such as the enteric nervous system, the vascular system, lymphatic systems and functional adaptive and innate immune systems. However co-culture protocols are being developed that allow numerous cells types to be grown at the same time. But, as more components are introduced, these compromise the development of the primary gut tissue.
Nevertheless, we will assess progress for outcomes that overcome these limitations. Thereafter, we can use these as testing ground prior to work in animals.

A retrospective assessment of replacement will be due by 16 May 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything

others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals estimated will allow us to carry out comprehensive studies to evaluate the efficacy of stem cell-based treatment for ischemic gut injuries. The investigations will involve examining the animals as well as tissues using a battery of tools which necessitate the provision of starting material for each study. The experiments have been designed to generate statistically significant data, which allows us to reach quantitative conclusions regarding the efficacy of the intervention.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Generally, advice will be sought from our Consultant Statistician, where appropriate, to ensure a statistical design that is used is efficient and minimises the number of animals required yet maintains sufficient precision and power. For example, advice will be sought when a specific pre-clinical model introduces large subject-to-subject variation.

Furthermore, a number of studies including our own have shown that the impact of IRI and NEC effect more than just the primary tissue under investigation, that being the gut. Therefore, in order for an intervention to be examined further for possible use in humans, it is now apparent that an intervention should be examine at the tissue functional level as well as in non-gut tissue, in particular the nervous system. We have developed a pipeline that extracts the maximal amount of data from each animal by not only determining gut function whilst the animal is alive, but then to isolate as many tissues as possible after the animal has been killed. This approach means that a single animal will generate data related to a wide variety of bodily functions.
We hope that the advent of robust and complex organoid based in-vitro culture systems will allow us to generate guiding and pilot data that allows us to conduct more precise experiments in live animals. For example when robust gut complex gut organoid protocols become available, we will carry out experiments to establish optimal doses of condition media before transitioning into animal based studies.
We will also use experimental protocols for both conditions that have been optimised over a number of decades for our studies. These protocols are the most robust yet and will have an impact on the number of animals being used. Again we will survey literature for developments that further refine them in-order to reduce animal numbers for a particular outcome.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The proposal aims to minimize animal usage by drawing on our extensive experience in this area of research. These insights will be deployed not only to keep the number of mice and rats to their lowest possible number but also to conduct the experiments in the shortest time span thus minimizing animal suffering.
A retrospective assessment of reduction will be due by 16 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

For both ischemic-reperfusion injury (IR) and necrotising enterocolitis (NEC) it is necessary to induce damage to the gut. Pain and suffering will be minimised through use of appropriate anaesthetic and analgesic regimes. The models selected provide the most data on paracrine mediated stem cell induced repair of tissue damage and will generate data for our company and others to develop definitive therapies while answering basic questions on stem cell biology.

Why can’t you use animals that are less sentient?

Small rodents (mouse: rat) are the simplest appropriate pre-clinical models to study these diseases and their potential amelioration. While there are species differences in physiology between humans and rodents, these are minimal and the use of species phylogenetically closer to humans is not required by the appropriate regulatory bodies and thus is not proposed as part of this project. Rodents are the animals of choice since they are amenable to surgical interventions whose outcome leads to tissue destruction very similar to IR and NEC.
Non-vertebrate models including nematode worms and fruit-flies do not share the same physiological parameters as humans and are thus not informative for this type of study.
Fish and amphibians have gastro-intestinal systems more similar to mammals than non-vertebrates. However, they are quite distinct to mammalian guts including major changes to the 4 distinctive layers found in rodents and humans. These changes could reflect their great reliance on an aquatic environment for their survival and differences in the diets. They are not good models for the diseases being investigated in this project.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The procedures to induce IR and NEC have been developed over decades and refined to delivery intended scientific outcomes with the least suffering to the animals. We will use the most optimised versions of these protocols available.
Importantly these protocols have been developed and refined, in order to best mimic the human condition and at the same time deliver this desirable outcome with the use of the smallest number of animals, by our partners who will be intimately involved in this project. These include some of the most eminent neonatal surgeons in the world. They will provide the expertise and oversight to this project. They will not only roll out the project but also ensure that all experimenters are fully competent in performing all aspects of the project.
As outline in our lifespan study, the use of paired animals (1 untreated: 1 treated) allows us to reach our endpoint (evaluating the impact of the intervention on survival) with the fewest possible animals.
For all surgical interventions, monitoring of the animals during recovery may be extended or increase in frequency if required. Furthermore, appropriate anaesthetic and analgesic regimes may be deployed to help

manage pain.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will seek to carry out the experiments using best practice advised by differing parties including:
Laboratory Animal Science Association (LASA) for best practice guidelines for administration of substances and genetically modified mouse welfare guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will seek advice from the Home Office inspector as well the named veterinary surgeon for alternative approaches using either less sentient animals or non-animal systems that allow us to achieve our goals.
A retrospective assessment of refinement will be due by 16 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

93. Investigate the role of physiological hypoxia and inflammation on cardiovascular disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Hypoxia, Cardiovascular disease, Endothelium, Pulmonary, Physiology

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall purpose of this project is to understand how the oxygen sensing pathway influences tissue and organ adaptations under physiological and pathological disease conditions.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The physiological responses to hypoxia (reduced oxygen availability), or to variations in the way tissues receive oxygen are complex and multi-dimensional. It forms a key component of many pathologies in humans and animals, and is particularly relevant to cardiovascular disease such as pulmonary hypertension, chronic obstructive pulmonary disease and myocardial infarction, where the restriction of blood flow leads initially to local tissue hypoxia (lack of oxygen) that if not quickly resolved initiates tissue dysfunction and potentially death.
A greater understanding of this response has the potential to aid drug discovery and target diseases including hypertension and COPD and heart attack.

What outputs do you think you will see at the end of this project?

One aspect of our work will look at the role of substances within cells which are involved in the regulation of oxygen which will
increase our understanding of the mechanisms involved which themselves are important with regard to the new generation of drug treatments for anaemia and cancer, currently in clinical trials. In particular, we hope to determine the role of a key substance and identify the mechanism of action for publication.

Pulmonary hypertension is a progressive and destructive disease of the blood vessels of the lung in which a substance known as HIF plays a key role. We will also determine the role of this substance which we hope to publish and also progress towards phase 1 or 2 clinical trail.
Our work will also focus on pulmonary hypertension cardiac arrhythmias which can shorten the life expectancy of patients. We have shown in pre-clinical models of PH that intervention with substances known as HIF2a inhibitors reduces many of the pathologies associated with this disease. This work will determine the role of HIF2a on cardiac arrhythmias in our pre-clinical models of PH. This will lead to a publication of new knowledge and contribute towards the progression of this drug to clinical trials.

Who or what will benefit from these outputs, and how?

This work will provide important mechanistic insight into the role of hypoxia and HIF2a in the mechanisms involved and the disease pathology of pulmonary hypertension. The data produced from this project will be presented at national and international conferences and published in academic journals. The knowledge gained from these investigations will also have wider implications in our understanding of acute pulmonary vascular disease pathology (bacterial sepsis, ARDS, acute mountain sickness).
The data gained will be shared with the pharmaceutical industry to advance the development of specific HIF2a inhibitors.

How will you look to maximise the outputs of this work?

The findings from this project will be made available to other scientists through publications in open access journals and presentations at scientific conferences and meetings

Species and numbers of animals expected to be used

- Mice: 3580
- Rats: 550

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will use both rats and mice.
We plan to use genetically altered mice that specifically targets the ability of the gene to produce a functioning product (known as gene expression) in the blood vessels of the lungs and allow us to investigate the role of our target genes in the development and progression of pulmonary hypertension. Modulation of these target genes in the lungs will prevent or accelerate the development of pulmonary hypertension. These mice have been specifically developed to target genes expressed in the blood vessels of the lung. The efficiency and specificity of gene modulation does not exist in other animal models.
We also plan to use rat models of pulmonary hypertension. These models offer a closer fit to human disease, closely matching immune cell function, lung remodelling and heart response observed in pulmonary hypertension. These models described offer the best pre-clinical to test new drug treatments for this lung disease.
We will use adult animals in both mice and rats, as they are immunological mature animals.
Typically, what will be done to an animal used in your project?

We all experience a degree of hypoxia (reduced/low oxygen) in our lifetime, whether this is self-induced (high altitude or extreme exercise) or disease originating (lung disease or cancer). Our overall objective is to obtain a better understanding of how and why our bodies specifically respond to low oxygen conditions with the view to using this knowledge to treat lung disease
We will use mice that have been genetically altered or novel drug treatments to improve our understanding of the normal physiological responses to acute and chronic low oxygen exposure. The body will normally rapidly respond to hypoxia by increasing respiratory rate, heart function and modify the method we use to generate energy (metabolism)
Acute hypoxia: The blood vessels in the lung respond to hypoxia by constricting (hypoxic pulmonary vasoconstriction-HPV). We will determine the role of our genes of interest in this highly conserved physiological response. Typically, mice and rats will receive our investigative drug or control vehicle by oral gavage (through a feeding tube in the throat) 30-90 minutes before the experimental procedure and hypoxia exposure. Mice and rats will be anaesthetised and a blood-pressure recording catheter inserted into the heart to measure HPV (blood pressure increases in the heart during HPV). The oxygen content of the air the mouse is breathing will be reduced to stimulate HPV. The pressure recording will take 30 minutes from start to finish. This is a terminal procedure, so the animal will not feel any pain. We will collect tissues and fluids for later analysis.
We will determine the effect on ventilation and metabolism by using non-invasive techniques. Mice or rats will be single housed inside these apparatus and will be exposed to the low oxygen environment for between 60-120 minutes.

We will also investigate how mice with diseased lungs respond to acute hypoxia and determine if the response is different to healthy lungs. We will use genetically modified animals that spontaneously develop pulmonary hypertension (3-4 months). The severity of the lung disease will be initially checked using a non-invasive imaging technique. These animals will progress through the sample procedure to measure HPV, respiration and metabolism as outlined above.

Chronic hypoxia: Typically mice or rats will receive our investigative drug or control vehicle by oral gavage twice a day for the duration of the protocol, or genetically altered mice for our genes of interest. These animals (mice or rats) will then be exposed to chronic hypoxia for a maximum of 35 days. During this time blood and urine samples may be collected, or respiratory rate will be recorded over a maximal 180 min period (non-invasive). We will assess heart function at the end of the hypoxia exposure via a terminal procedure. Tissues and fluids will be collected at this point for later analysis.

Models of Pulmonary hypertension: We will used both mice and rat models of pulmonary hypertension to determine the role of low oxygen, and hypoxia responsive genes in the initiation and development of pulmonary hypertension. We will use a combination of genetically altered mice and wild-type rats exposed to chronic hypoxia to initiate pulmonary hypertension. We will treat these animals (mice or rats) with our investigative drugs, administered twice a day for the duration of the protocol (21 days). We will use non-invasive methods to determine the progression of the disease and determine the change in metabolism. We also have the option to surgically implant a blood pressure measuring device (radio-telemetry) into a major blood vessel of the mice or rats. The animal (mouse or rat) is anaesthetised and receives an injection of pain relieving drugs before the catheter is carefully inserted into the blood vessel and secured. The transmitter is then inserted just under the skin on the flank of the animal. The wound is then sutured closed. The animal will quickly recover from the anaesthetic (5-10min) and surgical procedure over the next 7-10 days. This technique allows for blood pressure analysis over a continuous 28-days in conscious animals which is ideal for chronic hypoxia and drug intervention studies.

What are the expected impacts and/or adverse effects for the animals during your project?

For many of these experiments, the adverse effects are mild, in that animals (mice or rats) do not suffer undue stress by being subjected to the levels of oxygen employed by us experimentally. They will experience some increase in breathing rates and slightly lower levels of activity, much as is experienced by mountain climbers and other people at high altitudes. Some animals (mice or rats) will have surgically implanted units used to monitor parameters such as blood pressure and heart rate. When this is done, all procedures will be carried out with aseptic (sterile) surgical techniques and appropriate levels of anaesthesia, and pain relieving drugs will be administered during recovery from surgery.

Exposure to hypoxia: Manipulation of oxygen at the levels proposed are not expected to trigger adverse side effects, and the majority (>95%) of mice and rats tolerate these variations well up to 35 days. It's expected that mice and rats exposed to reduced oxygen levels undergo a mild weight loss due to decreased activity and metabolism, especially during the first week in the chamber. Both mice and rats will be weighted every week and those that show 15% of weight loss will be removed from the chamber and killed by a humane method.

Transgene inducing or deleting agents: In the majority of mice (>95%) no adverse effects are anticipated. Any animals that lose 15% of their body weight when compared to age matched controls will be promptly killed using a humane method.

Surgery: Surgical procedures will be carried out aseptically. In the uncommon event of post- operative complications, animals (mice and rats) will be humanely killed unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly. Peri- and post-operative pain relief will be provided. All animals (mice and rats) are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Animals will be closely monitored. Uncommonly animals that fail to recover by the end of the working day, exhibit pain, distress or significant ill health or otherwise deteriorate despite intervention will be killed by a humane method. Any animal not fully recovered from the surgical procedure within 48 hrs (eating, drinking and return to normal behaviour) will be humanely killed.

Diet alteration: Mice and rats generally tolerate a high fat or high salt diet favourably, Weight gain progress will be monitored weekly. However, animals will be humanely killed if they show signs of ill health, such as open fur-

coat and hunched posture, inactivity or inappetence for a period of 24h. Animals may gain up to twice as much weight as normal chow-fed animal over the same period of time (up to 3 months of high fat feeding). We do not expect significant weight gain or loss in the high salt diet, although daily monitoring of water use will be done to ensure that animals always have sufficient water. Previous studies have shown that high fat-fed animals feed, drink and move normally, may develop a greasy coat that the animals are not bothered by. However, if the weight gain appears to be inhibiting regular movement, or if the animals are observed to have lost appetite or are not drinking, the animal will be humanely killed immediately.
Substance administration and withdrawal of blood and/or urine: Most animals will undergo the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies as result some animals may experience no more than moderate levels of discomfort.

Plethysmography (measuring ventilation): Animals under this procedure are not expected to have any adverse effect other than initial distress when facing constraint within a chamber, observed by a failure to settle and that should disappear after 30-40 minutes, otherwise will be returned to its cage and tested other day. This will be reduced by conditioning in the chamber if necessary.
Housing in metabolic chambers: Animals housed in metabolic chambers supplied with 21% oxygen and free access to food and water are not expected to show any deviation from normal behaviour or well-being, as seen in their regular cages. If any, a transient exploratory behaviour will be observed when exposed to the new environment for first time. A small amount of bedding will be provided to help adaptation. In the unlikely event of clinical signs, such as open-fir coat and hunched posture for a period of 24h, the animal will be humanely killed. Upon exposure to low oxygen, animal will show a mild and transient increase in ventilation (no more than 20-30 minutes) followed by a drop in blood pressure and heart rate that will result in a reduced activity and loss of appetite for a period of 12 to 24h. Otherwise, both mice and rats adapt well to the oxygen levels proposed in this procedure and no further deviation from normal behaviour or wellbeing is anticipated. Animals will be humanely killed if showing signs of ill health, such as open fir coat and hunched posture, inactivity or inappetence lasting for a period of 24h hours. If mice and rats are maintained individually for more than 24 hours, they will be monitored extensively upon reintroduction to cages to ensure that no fighting or aggressive behaviour arises.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We estimate that 70% of mice will experience mild and 30% of mice will experience moderate severity and 60% of rats will experience mild and 40% will experience moderate severity.

What will happen to animals at the end of this project?

- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The response of cells to hypoxia differs from tissue to tissue, as well as within tissues themselves, and the fully evolved process cannot be modelled in any other system than a complete organism. Cultures grown in the

laboratory and even complex organoids (artificially grown mass of cells resembling organs) cannot replicate the complex interactions in the body that occur in hypoxia in tissues, or even the specific nutrient and mechanical environment in which they take place.

Which non-animal alternatives did you consider for use in this project?

We do use cell culture in the laboratory to test simple hypothesis and learn more about molecular pathways in the cell when feasible. Where possible, we do use all feasible methods to monitor and explore physiological changes outside of animal models. For example, we use cell co-culture systems wherever possible to model simple cell-cell interactions, and to validate functional and metabolic discrepancies. These co-culture systems are an important mechanism for replacement of in vivo (live animal) systems.

Why were they not suitable?

As discussed above, the response of cells, tissues and organs to hypoxia is incredibly diverse; we and others have identified hypoxic gradients across tissues and organs which influence physiological and pathological processes. These complex interactions between tissues and organs cannot be captured in laboratory culture systems.
We will continue to explore such systems for co-culture of multiple cell types to allow us to replace use of animal models wherever possible.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have a great deal of experience working with the animal models described in this project. We consulted with a trained statistician to ensure that our statistical monitoring is sufficient to minimize animal use

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The laboratory possesses and has access to highly refined equipment such as radiotelemetry receivers for monitoring of blood pressure, metabolic chambers coupled to oxygen mixers and detectors and whole-body plethysmographs, to accurately determine physiological outputs that ultimately will translate into reduced number of animals per group.
These models have been refined to enable us to maximise our data output from each animal. For instance, in the rat pulmonary hypertension model, each animal that has undergone a procedure will also have data on heart and liver metabolic profiles and tissues and fluids stored at -80C for future analysis.
All work carried out would be done in context of the NC3RS* ARRIVE** guidelines and framework to maximise research output whilst limiting unnecessary studies and use of research animals.
* National

* National Centre for the Replacement, Refinement and Reduction of Animals in Research

**Animal Research: Reporting of In Vivo Experiments

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use certain types of animals ('backcrossed lines') to avoid background variability that could potentially confound or mask phenotypes (the observed properties of the animal) deriving from genetic alterations, which will contribute to narrower and more specific range of observations, increasing the reliability of our results and therefore leading to reduced numbers of animals.
All of our genetically engineered mouse strains have been cryopreserved by sperm or embryo freezing. We will only produce mice in response to experimental demand.
Cell biology questions can and will be addressed using primary cell lines, purified from specific organs of interest, and these are used to guide our work in animals.
Collaboration with other groups at our institution, especially those undertaking different facets of cardiovascular research work, will allow for reduction of the total number of animals purchased and used at the institution.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are ideal models for the studies we proposed within this licence, for several reasons, including the physiological resemblance and ease of modelling the disease at hand, as well as the ability to genetically manipulate and expand new mouse colonies.
We have a great deal of experience running the methods described within this licence; these methods have been refined to cause the least pain and distress to the animal whilst maximising data collection and reducing group variability. We have developed non-invasive methods were possible, including whole-body plethysmography and metabolic analysis. Measurement of these physiological parameters occurs rea-time, so we use the earliest endpoint that allows scientific value. Mice will be otherwise closely monitored throughout the duration of any procedure.
We also use rat models of pulmonary hypertension. These models have been developed and refined as the gold-standard method to develop and test new potential therapeutic treatments. We will use non-invasive imaging to gauge the initial disease severity and track the development of pulmonary hypertension in these models. This close monitoring will enable us to complete the protocol at the earliest endpoint that allows scientific value.

Why can’t you use animals that are less sentient?

We aim to study how hypoxia/HIF signalling impacts on physiological and pathological disease processes. These studies required adult life stage for both mice and rats to ensure a mature and functional immune, cardio-pulmonary-vasculature to give the closest comparison with human physiology. Immature or embryo life stage mice or rats do not have a fully function immune system and/or cardio-pulmonary vasculature that is required to study cardiovascular disease processes.

The use of other vertebrate or invertebrate class is less than ideal to study the impact of hypoxia on physiological and pathological disease processes. The physiological structure/function of their major cardiovascular organs (lungs, heart, liver) are significantly different to that of humans, this would hinder direct comparison to pathological disease processes being investigated and negate any possibility of testing the stated hypothesis.

Many of the methods used in this protocol have been refined to be mild and non-invasive were possible.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All surgical procedures will be performed aseptically by trained surgeons, at least to the HO minimum standards for aseptic surgery, and according with the advice of the NVS. Pain relief will be provided during surgery and maintained after surgery for as long as necessary to alleviate pain. A close monitoring of post-operative animals will be undertaken.

We have a great deal of experience with blood pressure radio-telemetry, which allows us to monitor cardiovascular function for longer periods of time without mouse handling and/or restraint, reducing mouse stress and experimental variability. Improvements have been also been made in relation to the site and placement of the radio-telemetry device
Likewise, if new methods are developed, that are able to decrease animal distress or harm whilst maintaining accuracy and reliability, they will be evaluated.
All procedures that involve an alteration of normal mouse housing will be conducted after proper acclimatisation and training of the animals, as this increases consistency, diminishes variability and more importantly reduces the number of mice required to obtain statistically reliable results.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will keep up to date with the guidelines issued by LASA (https://www.lasa.co.uk) and NC3Rs (https://www.nc3rs.org.uk). These websites highlight a number non-invasive alternative protocols and promotes the advances made with new in vitro (laboratory based) techniques. (example https://www.nc3rs.org.uk/our-science/search-our-science?cells-systems%5B%5D=248)

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Many of the methods described in the project are the gold-standard for animal welfare and quality of data collection. The companies that developed this equipment are constantly refining and fine tuning their capability. We will keep on top of technical advances in this area of research, for instance Data Sci have now developed a radio-telemetry probe to constantly measure blood glucose concentrations. We will assess the benefits of this methodology and new developments in the field before progressing with the described protocols in this project. The LASA website highlights a number non-invasive alternative protocols and promotes the advances made with new in vitro techniques.

Home Office

NON-TECHNICAL SUMMARY

94. Investigating blood development

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Stem cells, Haematopoiesis, Zebrafish, Single-cell Transcriptomics, Blood

Animal types	Life stages
Zebra fish	adult, embryo, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand which genes are important to maintain the correct number of different blood cell types in a normal environment and under immune challenge.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the

duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

With the extraction of information from single cells, we will identify key genes and pathways that play a role in making blood cells. The results from this work will inform the ways to grow blood cells in vitro for use in regenerative medicine and will increase our understanding of mechanisms underlying blood pathologies.
What outputs do you think you will see at the end of this project?

This study will generate a number of valuable experimental and computational tools that will be made freely available to the scientific community. For example, we generated new computational tool to identify different cell types and genes that are necessary for development of these cell types.
Furthermore, we generated a user-friendly data storage for interactive exploration and visualisation of single-cell data from blood and immune cells in zebrafish.

The results from this work will inform the ways to grow blood cells in vitro for use in regenerative medicine and will increase our understanding of mechanisms underlying blood pathologies. We will develop a user-friendly website which will allow researchers to access and understand our data. Our project will open up a new field of research in lower vertebrates (e.g. killifish and other fish breeds) and is designed to allow other researchers to build on our achievements. Our website will allow researchers bringing expertise from related fields but without our background knowledge to immediately begin productive work.

We will present our research at a range of leading academic conferences to engage researchers from multiple fields in our research and ensure follow on use of the data made available through our website. We will also present at major conferences for commercial fisheries, e.g. Aqua-Fisheries.

We will present our research in high impact peer reviewed journals. This will ensure wide exposure for our research and encourage other researchers to further exploit our findings.
Who or what will benefit from these outputs, and how?

As this project tackles important basic questions, it has relevance to many people or groups who support and fund our research. Researchers will gain important insights into blood development and better understanding of the processes involved in blood diseases. Furthermore, our work would be of interest to those concerned professionally with breeding fish and fisheries, government departments concerned with food security, and the wider public interested in animal welfare, ecology, the food chain and broader scientific questions. These benefits would not be direct, but instead a long-term indirect benefit that will arise from the fundamental data our research will generate These researchers and industries will benefit because of the discoveries our research will lead to as we are studying the immune system of fish and its response to germs.
How will you look to maximise the outputs of this work?

We will maximise our output by presenting our work and ideas at national and international leading conferences. We will present both, positive and negative results to inform the research community of the drawbacks of different techniques and approaches.

Additionally, we will continue to work with researchers in the field which will enable the exchange of knowledge, ideas and techniques. This would maximise the quality of research in the field.

Species and numbers of animals expected to be used

- Zebra fish: ~11,200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Each day human body generates more than 200 billion blood cells. All these different blood cells perform various functions such as clotting of blood, fighting germs and carrying oxygen. Importantly, all these different blood cells also exist in zebrafish and perform similar functions. In this project we would like to understand how are these different blood cell types generated and how are their numbers regulated. To do this it is essential that we study these cells in their natural environment i.e. in the body of the fish. The system that uses cells made in a dish does not resemble the environment in which different blood cells are developing and functioning. To understand their exact function and origin, it is necessary they are studied in the context of the whole body as opposed to being studied in a dish.

Typically, what will be done to an animal used in your project?

For our first objective, fish will be killed using an excess of the anaesthetic and their blood cells will be isolated from different organs. We will then study the genetic material of these blood cells. The fish used could be at the embryonic, juvenile or adult stage. For our second objective, we will use the information we gained from studying the genetic material of blood cells and create animals where we alter their genes. These animals will be used to isolate distinct blood cells from zebrafish and to learn how different genes affect blood development. For this process, we will take blood from these fish and inject them into another fish i.e., recipient fish. Recipient zebrafish will be exposed to radiation and an injection of donor blood cells under anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of fish (94%) will experience no side effects, 5% of fish are expected to experience mild effects such as being unable to maintain skilful and effective coordination of movements. A small proportion of animals (under 1%) are expected suffer moderate effects such as reduced food intake and being unable to maintain skilful and effective coordination of movements.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of fish (94%) will experience no side effects, 5% of fish are expected to experience mild transient effects. A small proportion of animals (under 1%) are expected to suffer moderate effects.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

For developmental purposes, it is the interaction of different cell types in the blood of intact animal that controls most developmental choices. As we are intending to understand the function of genes that currently have no known function, it is essential to have every cell type of the whole animal present to detect the function of genes.
Which non-animal alternatives did you consider for use in this project?

After attempting to work with cells in a dish, we determined that dish does not resemble the exact environment in which different blood cells are developing in a body. Therefore, we need to use animals to fully understand how different blood cell are made. We will, however, use artificially grown blood cells from human to extend some of our findings and observations from zebrafish to human system.
Why were they not suitable?

They failed to produce the blood cells of interest which made it impossible to understand their origin and function.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

As my laboratory has been using the zebrafish model system for the last 8 years, based on previous data generated we estimated the number of animals that we will use.

We estimated ~11,200 would be sufficient to perform all the experiments and achieve the objectives outlined in this project license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To reduce the number of animals kept in the fish facility we aim to maintain the fewest number necessary to carry out a particular experiment by using computational methods. Furthermore, we use online tools such as NC3R's as well as PREPARE guidelines when designing an experiment.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In order to reduce the number of fish grown for experimental purposes, we standardised our protocols for clipping embryos at 3 days post fertilisation (dpf). The embryos are sorted as per their genetic makeup (before 5 days after they are born) and only the fish relevant for our research are raised to adulthood. This method reduces the number of fish being grown into adulthood.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

After attempting to work with an in vitro system (to grow cells in a dish), we determined that an in vitro system does not resemble the exact environment in which different blood cells are developing and therefore, would not be suitable for identifying cellular states that underlie blood development. We will, however, use in vitro single-cell differentiation assays of human stem cells to extend some of our findings and observations from zebrafish to human system.

We will be using zebrafish as a model system as these animals are less emotional compared to other model systems used in blood research (e.g. mice, rats, sheep, pigs, etc). We will preserve lines that are not currently in use by freezing the sperm of the male adult fish, thus only maintaining actively used animals in the facility. We will also re-use fish for gamete recovery which in turn reduces the total number of animals in the project.

Why can't you use animals that are less sentient?

We cannot use animals that are less emotional than zebrafish (e.g. fruitfly) as they do not possess all blood cells that human have.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will continue monitoring our fish daily by observing for any unexpected movements or behaviour of the fish (e.g. rapid opening of the mouth at the water's surface, unable to maintain skilful and effective interaction of movements, development of lesions). Additionally, after any time something is done to an animal it will be closely monitored for any signs of pain or distress so an early intervention can occur to stop this.

Fish receive brine shrimp and paramecia for enrichment. All fish are housed in male and female groups where possible to reduce egg bound females and ensuring natural behaviours. Breeding tanks are tilted to replicate a more natural environment. For example, actions such as waiting and circling are observed in a natural environment, but not in a lab due to the unnatural characteristics of the mating tank. By tilting the tank we mimic the bank of the Eastern India's Ganges river where zebrafish were originally found. The fish that are being identified for their genes are housed separately and are kept on the system so that they are able to receive food and fresh water.

In order to reduce the number of fish reared for experimental purposes, we standardised our protocols for cutting the tip of the fin of embryos 3 days after they are hatched. The embryos are sorted as per the gene they possess (before 5 days after they hatched) and only the required genotype (genetic makeup of a cell) are raised to adulthood. This method significantly reduces the number of fish being reared as genotyping at 3 days after they hatched eliminates the need to raise 100s of fish in order to genotype at 3 months of age. This is the main route that will be taken to genotype our fish. However, there will be occasions where it will not be possible to genotype our fish at 3 days after they hatched. For this reason, some fish lines will have to be genotyped at 3 months (adult stage), where a biopsy of the caudal fin (tail fin situated at the rear of the fish) will be taken. The caudal fin is used because there is no circulation and no nerves, therefore the procedure is painless for the animal. In conclusion, some fish will be finclipped at 3 days after they hatched, some will be genotyped at 3 months of age. Some fish will be phenotyped via fluorescence and in cases two fin clips will have to be done. Fluorescence is used for phenotyping fish (observable characteristics) that have their blood cells labelled with fluorescent protein. This fluorescent protein is part of genetic makeup of these fish and its fluorescence is used to select fish of interest in a way that is painless for the animal. All efforts will be made to ensure that unnecessary breeding of fish will be kept to the minimum.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will continue using the NC3Rs website and keep up to the date with the PREPARE guidelines to ensure experiments are conducted in the most refined way in addition to following animal welfare information through the Laboratory Animal Science Association (LASA) website. Furthermore, we will also follow guidance on the housing and care of zebrafish
https://www.rspca.org.uk/webContent/staticImages/Downloads/HousingAndCareZebrafish.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in the 3Rs through newsletters received from the institute's aquatics facility as well as routinely checking the NC3Rs website. Additionally, advice and up to date information will also be obtained from the institute's named persons (e.g.: NACWO, NIO and NVS).

Home Office

NON-TECHNICAL SUMMARY

95.Investigating the efficacy and potential of a novel delivery system to transfer materials across the blood-brain barrier

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Rats	adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim is to use a novel delivery system to transfer abnormal proteins across the blood-brain barrier, to generate a more refined animal model for Parkinson's disease. The same delivery system could also be used to transfer compounds of therapeutic potential or imaging capability to the brain, to facilitate the development of drugs and biomarkers for several neurological conditions like Parkinson's disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

One of the main hurdles to brain research is the difficulty of bypassing the blood-brain barrier to facilitate the delivery of substances into the brain to be able to model neurological conditions, drugs to alleviate relevant clinical features, and biomarkers to track disease progression over time. Using Parkinson's disease as an example, this age-related neurological condition is defined by the presence of motor problems that are associated with the loss of a specific group of nerve cells in the brain producing a chemical called dopamine. Conventional animal models can be generated by specifically killing these dopamine-producing cells to reproduce motor abnormalities. Recent advances in Parkinson's research have however highlighted an important role of a protein called alpha-synuclein, which can cause many of the motor and non-motor symptoms when it starts to clump together abnormally. The blood-brain barrier crossing efficiency of the abnormal alpha-synuclein protein, as well as compounds designed to deter their formation, is very poor, meaning that they currently need to be delivered directly into the brain by surgery, thereby limiting their value in neuroscience research. To address this issue, in this programme of work we will harness the versatility of a novel delivery system to validate its capacity to deliver a variety of materials, such as in the first instance abnormal alpha-synuclein, plus drugs and biomarkers that can potentially be used to alleviate or to track alpha-synuclein pathology, into the brain following peripheral administration. In future we feel that this method of delivery into the brain could have much wider applicability.

What outputs do you think you will see at the end of this project?

The main limitation to develop better medications to help Parkinson's disease patients is the lack of good experimental models that fully reflect human pathology. At the end of this project, a more refined animal model of Parkinson's disease will be optimised and made available to the research community through open-access publications in academic journals. In addition to this, several novel therapeutics, repurposed drugs (medications that are approved by medical authorities for human disease conditions, but are not specifically licenced for Parkinson's disease), and novel imaging agents will initially be trialled in our cell models. These drugs and imaging agents that are verified in the lab will then be evaluated in our animal models in the hope to assess their efficacy in minimising the effect in the brain and behavioural functions that are related to Parkinson's disease. Data regarding the efficacy and tolerability of these agents will also be made available to the research community as well as to the general public.

Due to the limited funding currently available for this project, by the end of the current funding period we would have finished characterising this refined animal model of Parkinson's disease to aid further therapeutic development. Therefore, the identification of novel therapeutics, repurposed drugs, and potential imaging agents will be conducted in the lab in parallel to the characterisation of this refined animal model. We are, and will continue to actively seeking for further funding support, in order to be able to evaluate such agents identified

from our cell models in our newly refined animal model.

Who or what will benefit from these outputs, and how?

Research output from this programme of work will directly benefit scientists studying how the disease unfolds in the course of Parkinson's disease, scientists who wish to validate if their experimental therapies can help the disease in a relevant animal model, or clinicians planning to conduct further clinical trials to assess cell therapies in Parkinson's disease. Such data will be made available to the public through open-access academic publications.
For the drug screening and the development of imaging agents: any favourable outcomes indicating the safety and effectiveness in the animal model generated can potentially be translated to help Parkinson's disease patients in the future. My group is based in a research environment that is very experienced in conducting clinical trials in Parkinson's disease. It is therefore possible that research output from this project can be transferred to the clinical domain by the end of the 5 years period of this licence.

How will you look to maximise the outputs of this work?

Research output will be communicated through a number of routes to a range of audiences - including scientists, clinicians, as well as patients and the general public.
Scientifically, the work will be communicated primarily in the form of academic publications in open access journals as well as collaborations at the local, national, and international levels. Research outputs will also be distributed in national/international meetings, including those meetings which are organised by specific charities such as Parkinson’s UK or Cure Parkinson’s trust in UK. The work will also be presented in seminars both locally (within our own department and others within the University/Hospital), as well as in other institutions.
We will also communicate our work openly to the patient community through our social media outlets (e.g. the lab Facebook page), our annual newsletter that is sent out to all patients and their families attending our research centre, as well as our annual open days for patients.

Species and numbers of animals expected to be used

- Rats: 550

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In most cases conditions that affect the brain, for instance Parkinson's disease, are not transmittable from parent to offspring, and does not pass down through families. Therefore, to model reliably the symptoms of this condition in animals, we need to use animals that sufficiently reflect the difference of genetic make-up between individuals. Animals known as ‘wild-type’ and ‘out-bred’ have this variability in their genes, and can therefore better represent the diversity that people have with each other and are best suited for modelling Parkinson's disease. Sprague-Dawley rats are a wild-type out-bred strain and are the best animals to use for this purpose.
It is now believed that changes in the brain cells in Parkinson's disease can happen many years before symptoms that affect the movement emerge, therefore in this programme of research animals will be lesioned at 3-4 months of age, and followed over time to track disease progression over the next 6-18 months. In addition, ageing is known to be the main risk factor to developing this condition, and on average symptoms of Parkinson's

disease are rarely observed in people younger than 50 years of age. Therefore, in some experiments lesion will take place at a later stage of life (e.g. 12 months of age), with close follow-ups over the next 6 months to determine if disease progression is accelerated.
On the other hand, for experiments that are designed to test cell therapies, rats that lack a fully functional immune system (such as the RNU rat model) are best to use as their immune system would not attack the newly implanted cells. These rats have been bred specifically for this type of work and lack a functioning immune system; were normal rats to be used they would require daily injections to suppress their immune system. However, in other experiments the use of wild-type rats with daily immunosuppressant injection is still required, as this will better reflect the daily injections given to Parkinson's disease patients after receiving cell therapy.
For the type of work outlined in this research programme, rats are better animals to use. There are several reasons for this. Firstly, rats are mammals and compare with using invertebrates that have a very different nervous structure, the use of mammals to develop a refined model of Parkinson's disease will minimise the risk of future translation into human studies. Secondly, compare with mice, rats are known to be less prone to stress through handling as they are more likely to create a bond with a single handler. This is beneficial as they will be kept and followed for a long time period. To reduce stress further, they will be handled by the same researcher whenever possible, as well as being housed in the same environment as they will have appropriate time to get used to it. Finally, rat brains are much larger than mouse brains, and would therefore provide larger and clear images for data analysis.

Typically, what will be done to an animal used in your project?

In a typical experiment, adult rats will be given an injection of a pathological substance (which is a substance that induces disease or illness) via the tail vein (and occasionally into the brain as well), with the intention that the disease will emerge and progress over the next 6-18 months (depending on which protocol is used, as some protocols allow for animals to be kept until 2 years of age and others only until 15 months of age). Over this entire period, animals will be followed closely for any signs of deterioration of their movement and memory functions using behavioural testing, in order to determine the optimal period for giving medications to alleviate the symptoms. These medications will be identified in the laboratory using cells grown in a dish, and will then be given to the lesioned animals through routes of administration appropriate for individual medications. This includes delivery via food or drinking water, injection at regular intervals, or those that have to be delivered directly into the brain such as cell therapies. In order to track disease progression over time, some of the lesioned rats will be followed by preclinical imaging analysis, including positron-emission tomography (PET) or magnetic resonance imaging (MRI), in order to help develop biomarkers (an indicator of a particular disease state) to monitor the changes in their brain function over time. By the end of the experiments, animals will be killed humanely to correlate behavioural changes with disease features in the brain.

What are the expected impacts and/or adverse effects for the animals during your project?

For the majority of the animals we anticipate no or very little adverse effects to occur. However, after the disease has started and as the animals age during the experimental period, we expect to see clinical signs that are similar to those seen in Parkinson's disease patients. These include: reduced motor activities, reduced gripping capacity, suppressed limb coordination, abnormalities in the pattern of movement, and memory dysfunction. They may also incur adverse effects not associated with the disease itself but those relating to the ageing process, such as tumour formation (most commonly benign mammary tumours in rats) or weight loss. Animals will be closely monitored with veterinary involvement, and animals will be killed if weight loss reaches 15% or the position of a tumour reaches 40mm or affects behaviour such as normal movement.
In addition, some animals will undergo surgery, this may create discomfort in the immediate postoperative period, during which time pain relief will be provided. They may show reduced or altered movements after surgery, where they have received a lesion (cell damage) to mimic an aspect of the human disease. These animals may later receive a treatment to repair the lesion given, such as cell transplantation or drugs that we have tested in the lab. Animals are expected to have mild to no lasting adverse effects after they have recovered

from the surgery itself. Treatments are also likely to result in no adverse effects but if any should arise, they will be monitored for up to 24 hours, in close liaison with the vet. If no improvement is seen during this time then the affected animals will be killed humanely.
In order to study the Parkinson's disease-like clinical signs in our animals, some of them will undergo food restriction to motivate their performance in behavioural tasks which are reward-driven, and they will be carefully monitored to ensure that no sign of dehydration occurs, and any weight loss is kept within a defined limit. We expect no adverse effects associated with the behavioural tests used in this study.
At the end of study all animals will be killed humanely, and tissues will be taken for further investigation in the laboratory.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Only adult rats will be used for all experiments.
For those experiments that are related to disease modelling, most animals (more than 50%) will experience a mild degree of severity. Some animals (less than 50%) will experience a moderate degree of severity that is related to either the ageing process itself (when animals are kept for longer than 15 months of age), or related to the procedures performed on the animals.
For those experiments that are related to treatment most animals (more than 50%) will experience a mild degree of severity. Some animals (less than 50%) will experience a moderate degree of severity that are related to the treatment, such as the injection of cells directly into the brain, or repeated administration of medications at regular intervals. No aged animals will be used for trialling treatment.
For those experiments that are related to testing imaging agents for the first time in animals, animals will experience a mild degree of severity when given the disease lesion but the administration of the imaging agents will be done using a procedure where the animal doesn't wake up. This is to assess the impact of these imaging agents on vital signs of life, without causing unnecessary suffering of the animals. Once considered safe these imaging agents will then be used in the other protocols.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The blood-brain barrier is a highly selective border that separates the circulating blood from the central nervous system. There is currently no cellular model of the blood-brain barrier that provides sufficient level of "tightness" and "selectivity" to study the novel delivery system. Furthermore, the risk of developing Parkinson's disease increases with age. While the use of cell cultures can provide important insights into studying particular aspects of this condition, they can only reflect the early stages of development. It is therefore essential for Parkinson's research to use animals to model the disease condition, to be able to look at the adult/aged nervous systems that are affected by the disease. Another main reason is that Parkinson's disease is defined by the presence of behavioural abnormalities. It is very difficult to study how cells respond to the disease condition in the "behavioural" domain, driving the necessity of the animal model to be able to model accurately and analyse the disease.

Which non-animal alternatives did you consider for use in this project?

My group is currently using cellular models to facilitate the screening of drugs and useful cell therapies, including those that are already licensed for use in the clinic but not for Parkinson's disease. This will help minimising the number of animals we need to use for our research, as only drugs with proven protective activities will be brought forward to animal testing. This same cell culture system will also be used to aid the development of novel markers to track disease progression in animal studies. In addition, we use post-mortem brain samples donated by Parkinson's disease patients and healthy individuals to understand the disease features in the brain. A cross reference between cellular models, post-mortem analysis, and animal research will help ensure the data we generate will be useful to reflect the conditions experienced by Parkinson's disease patients, and will therefore facilitate the development of new drugs.

Why were they not suitable?

As indicated above, we are currently using cells in vitro and expose them to toxic proteins that are known to play a key role in Parkinson's disease to model this condition. However, these cells lack the "ageing" component, which is the main risk factor to the development of Parkinson's disease. We are also using post-mortem brain tissues to verify our findings in cellular models. However, it is impossible to test treatments fully in non-living tissue. Computer modelling is used by some to look into modelling the disease but anything that is found has to be verified using animals prior to use in humans. Therefore, the use of animal models of this disease condition and in which to test new drugs remains unavoidable.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals used in this project is estimated from our previous experience on data analysis, which provides information to predict the minimal number of animals required for each experiment. We also take into consideration the number of experiments we expect to perform, based on the projected funding availability. We have access to statistical advice and will seek it whenever necessary.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have conducted pilot studies under our previous project licenses to obtain preliminary data on the degree of behavioural deficits expected. Such information is then computed using GLIMMPSE <http://glimmpse.samplesizeshop.org> for calculating sample size for repeated measures and longitudinal designs. Further advice on statistical design and analysis can be obtained easily in the establishment, for instance through the establishment's statistics clinics, run by the Faculty of Mathematics which provides a free, walk-in statistical consulting service to university members. Similarly, there are biostatistical assistance provided on-site, to whom I can seek help when necessary. The use of the Experimental Design Assistance from the NC3R website <https://www.nc3rs.org.uk/experimental-design-assistant-eda> will be adopted whenever appropriate. Similarly, animals will be randomised and the experimenter will be blinded when analysing the results, so accurate information can be obtained without bias.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In addition to the use of pilot studies to generate data for statistical analysis, the animals will be followed longitudinally to reduce individual variance. The use of animals will be further optimised by employing preclinical imaging, such as the use of positron-emission tomography (PET) or magnetic resonance imaging (MRI), as well as tissue sharing (such as repeated blood sampling), in order to study disease progression over time.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In this project we will use the novel delivery system to model Parkinson's disease in two ways: (a) by delivering toxic proteins, such as an abnormal form of alpha-synuclein, into the circulation by directly injecting into the tail vein; and (b) by combining the peripheral injection with a direct injection of other toxic chemicals into the brain to more kill the dopamine-producing effectively in the brain. The delivery of these substances through the tail vein will create less suffering and distress to the animals compared with other methods typically used. However, a direct brain injection will occasionally be required to either generate a more substantial level of cell loss, or for treatments that cannot be administrated by other means (such as cell implants). Cell implants are made into areas of the brain where cell damage and death has been caused previously and the intention is for the implanted cells to repair the damage previously made. Where surgery is performed it will be undertaken using strict aseptic surgical technique to minimise the risk of infection, and the animals will be closely monitored before and after the procedure, in order to minimise the level of suffering and to ensure there is no lasting harm to the animals. For all surgical procedures, animals will be given pain killers to minimise the pain they may encounter when they recover.

Why can’t you use animals that are less sentient?

To study a novel delivery system that can facilitate the transfer of materials across the blood-brain barrier, we will need to use a mammalian species that has an intact blood-brain barrier and has been established as a research model for this purpose.
In addition, as the risk of Parkinson's disease increases with age, it is relevant to study the disease and develop novel treatments that affect a mature/aged nervous system. Furthermore, Parkinson's disease is a long-lasting condition that progressively evolves, it is therefore essential to monitor behavioural changes in live animals over time. The rat model we want to generate is therefore the least sentient beings still able to mimic Parkinson's disease. When using non-mammalian species, the issue that will arise is that any novel treatments found cannot be directly translated into a human setting.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Harm to the animals will be minimised by the use of the least invasive (e.g. tail vein injection versus brain surgery) route of administration to deliver the substances that induce the Parkinsonian features wherever possible. With help from the establishment's biofacility staff, our animals will be closely monitored on a daily

basis so that any signs of pain and/or distress will be dealt with in a prompt and effective manner. Animals will be weighed at regular intervals and signs of any adverse effects will be monitored in direct liaison with the vet, to ensure that any welfare costs are kept to the minimum.
For the administration of novel compounds, pilot studies involving only a small number of animals will be tested first. These experiments will also be done using a procedure in which the animal doesn't wake up but their vital signs of life can still be assessed, in order to examine the tolerability of these novel compounds to the animals. This will determine the optimal dosage without creating unnecessary welfare costs to the experimental animals.
Animals will also be housed in groups whenever possible, with environmental enrichment provided after discussion and recommendation from the Named Animal Care Welfare Officer and senior technicians from the establishment's biofacility.
If the Parkinson's disease model created shows definitive clinical signs in animals over time, then an assessment/scoring system will be developed to refine the humane endpoints for future projects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Experimental design will be done in light of the newly published PREPARE guideline, as well as using the NC3Rs Experimental Design Assistant <https://www.nc3rs.org.uk/experimental-design-assistanteda> wherever appropriate. Delivery of substances directly into the brain will be done using aseptic techniques, in line with the LASA Guidance on Aseptic Surgery. All the experiments will be conducted, with the results analysed and reported in accordance with the ARRIVE guideline.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our primary source of 3Rs information will be the NC3Rs website. Researchers involved in this project have access to the establishment’s biomedical services website and receive regular 3Rs updates. More specific advice can be found on the 3Rs search tool on the establishment’s biomedical service website, which provides an abundance of 3Rs information from worldwide websites. Finally, researchers involved in this project will also have constant dialogues with the biofacility staff in order to stay informed about effective implementation of the 3Rs. Such advances will be done in liaison with the Named Animal Care Welfare Officer, vet, and biofacility staff, throughout the duration of this project.

Home Office

NON-TECHNICAL SUMMARY

96. Investigating the mechanistic drivers of neuropathic pain and treatments to reverse them

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Neuropathic pain, Sensory biology, Chemogenetics, Analgesia

Animal types	Life stages
Mice	adult, juvenile, neonate, pregnant, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We do not have a clear understanding of the how the nervous system is organised to facilitate the sensation of touch, temperature, itch and pain. We aim to define the contributions of different classes of primary afferents (the nerves responsible for detecting sensory stimuli) and how their signals are integrated in the spinal cord. This work will underpin our concurrent studies seeking to define how changes in this processing results in neuropathic pain and testing treatments to reverse these changes.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Neuropathic pain results following damage to the nervous system and is a major burden on society, affecting roughly five million adults in the UK. The majority of patients live with chronic and often debilitating pain because current medications are ineffective. Even the most effective analgesic only provides clinically relevant pain relief to 1 in 5 patients. Only when we have a good understanding of how the nervous system is organised to signal normal and neuropathic pain, will we be best placed to design more effective pain-relieving medications.

What outputs do you think you will see at the end of this project?

Work completed under this license will provide information on how we are able to detect sensory signals such as touch, temperature, itch and pain. It will also reveal what goes wrong in this system in the context of nerve injury and the changes which occur that lead to neuropathic pain. Finally, we will test the effectiveness of novel approaches to reverse such changes and their potential as analgesics. Outputs will take the form of publications in peer reviewed journals, abstracts at national and international meetings, and technology development.

Who or what will benefit from these outputs, and how?

In the short term, outputs will have a major benefit for other researchers in the pain field. Our data will allow research and translational efforts to be focussed on the parts of the nervous system that are driving neuropathic pain. During our studies we will test novel approaches for the reversal of pain and so our outputs will benefit drug discovery programmes of biotechnology and pharmaceutical companies. In the long-term, the consequence of both of these benefits will be to improve patient care and meet the clinical need of neuropathic pain.

How will you look to maximise the outputs of this work?

We will maximise outputs of this work by collaborating with other groups in the field of pain research.
Data will be published in international peer reviewed journals and data will be freely provided with an "Open Access" ethos. Unpublished data will be presented at national and international scientific meetings to ensure that it is available to other researchers in the field. We will also participate in open days and other forms of public engagement, to disseminate our work and contribute to public awareness.

Species and numbers of animals expected to be used

- Mice: 7500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse sensory nervous system has been demonstrated to be comparable to human in most tested aspects and there is a large pre-existing knowledge base which will be used to maximise the outputs from this project. Mouse models of neuropathic pain are commonly used and have been designed and refined for clinical relevance. The sensory disturbances that they exhibit mirror many features of human neuropathic pain patients, including exaggerated responses to evoked-pain and spontaneous pain. We will turn off the electrical activity of specific nerve populations to understand the role they play and require genetic strategies to do so accurately. This is only available in a few organisms, of which the mouse is best placed due to the previously mentioned reasons. Neuropathic pain occurs in patients of all ages prevalence increases with age. Therefore we will perform studies with all ages of animals.

Typically, what will be done to an animal used in your project?

The majority of animals will not undergo any procedures which result in adverse effects as they will be born in the process of generating experimental animals. Experimental animals will typically undergo a short surgical procedure to deliver an agent which can silence specific groups of nerves. They will then undergo reflex withdrawal testing of their sensory perception while silencing is activated, in order to ascribe function to the silenced nerves. In some cases, testing will occur when the animal is in a neuropathic state; induced by lesion of a peripheral nerve, diabetes, or administration of a chemotherapeutic drug. In the majority of cases, the time from delivery of the silencing agent to humane killing will be 6 weeks. In cases of delivering agents to neonatal animals, or in studying the long-term consequence of nervous system damage, studies may take up to 14 weeks. However, these cases will be rare and kept to a minimum.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of animals will not experience adverse effects. Neuropathic experimental animals will experience sensory disturbance consistent with the human experience. These include hyper- or hyposensitity to sensory stimuli and a degree of ongoing pain in some of the models. These do not impact the behaviour or general wellbeing of the animal in terms of feeding or grooming and will be time limited (4 weeks in the vast majority of cases). Models of Type 1 diabetes and animals which receive chemotherapy agents can suffer from weight loss, which we will monitor for very carefully. Surgical procedures may induce transient discomfort (up to 24hrs), but we will provide analgesics to provide pain relief.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild- 70% of animals

Moderate- 30% of animals

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animal use is necessary to address the functional roles of primary afferents during normal and neuropathic pain. Microneurography studies in humans have defined the response properties of classes of afferents in healthy patients and those suffering from neuropathic pain. They have, for example, been used to demonstrate that following nerve injury, excessive primary afferent activity occurs which correlates with the pain experienced by the patient. Limited interventional studies have also been performed, such as using local anaesthetic nerve block to demonstrate that this excessive activity is critical for pain. However, this approach is not suitable to define the contributions of precise nerve sub-populations and therefore we require use of genetically modified rodent models, in which discreet nerves can be targeted for manipulation and studied.

Which non-animal alternatives did you consider for use in this project?

There exist several replacement alternative experimental models which we will take advantage of, but which cannot be used to fully replace the animal studies.

Primary afferents derived from mouse sensory ganglia and cultured in vitro represent a useful experimental tool and a partial replacement of animal studies. We will use these where possible to define key molecular pathways that contribute to primary afferent signalling and to assess the consequence of treatments to alter intrinsic properties.

Induced pluripotent stem cells (iPSC) differentiated into human primary afferents, represent a powerful cellular model that replicates many of the features of endogenous primary afferents. As a key component of our work is to translate novel analgesic treatments, we will use these cells as a platform to validate findings across species and to provide a replacement of animals where appropriate.

Why were they not suitable?

Normal and pathological sensory perceptions are complex phenomena and involve the whole sensory nervous system axis; spanning the detection of sensory stimuli in the periphery, to the conscious perception in the cortex. As such, certain features can only be adequately assessed in vivo. Many of the relevant processes involve multiple connections of nerves and interaction with other cell types, which cannot yet be modelled in the in vitro systems described above. Primary afferents are highly heterogeneous, and while iPSC-derived human primary afferents model select sub-populations well, protocols do not exist to derive the full diversity present in sensory ganglia.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals optimally required for each aspect of the study have been added together. These calculations underpin our estimate of usage and numbers are in line with our previous experience of similar studies.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experimental design and data analysis was discussed with a local statistician prior to drafting of this application. The Nc3Rs Experimental Design Assistant was used to design some experimental paradigms.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To optimise the number of animals used, as much work as possible will be undertaken in cellular models before moving to animal models. For example, we will screen the effectiveness of delivery systems and novel therapeutic agents in stem cell derived neurons to ensure that they function as we would expect. This approach will help remove ineffective approaches from testing in animals. We will ensure optimal breeding strategies of transgenic animals in order to reduce the number of animals bred of a genotype not required for experiments. We will openly share tissue and data with other laboratories working in the field of sensory neuroscience to ensure that animal experiments are not replicated across locations.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mice which allow us to target silencing agents to specific groups of nerves, in order to study the roles of these nerves. None of these animals will experience any adverse harmful effects as a consequence of the genetic alteration that they carry. In the majority of cases, animal sensory perception will be measured by testing of reflex withdrawal thresholds i.e. at what level of a stimulus does the animal withdraw their hind paw? By halting the stimulus as soon as a withdrawal is made, we will ensure that the minimal level of stimulus is used to characterise sensory perception. Some mice will be used to experimentally model neuropathic pain. Such manipulation may occur in a variety of ways. Genetically altered animals with a predisposition to develop diabetes (and consequently, painful diabetic neuropathy) are a great refinement on classical toxin-based models of diabetes and result in less severe hyperglycemia, do not include the direct neurotoxic actions of drugs and therefore much better model human diabetes. Some animals will be treated with chemotherapeutic agents to mimic chemotherapy-induced neuropathy. In all cases, agents will be used at doses which induce the neuropathy, but are limited for their impact on the general wellbeing of the animal. Finally, some animals will undergo surgical lesion of nerves, to model traumatic nerve injury induced neuropathic pain. Such surgical interventions are well established models which have been refined for decades and across many laboratories, to best replicate the human condition and refined to minimise animal suffering. In all cases, we will monitor for any evidence of undue animal suffering and act swiftly to minimise this, in most cases by humanely killing the animal.

Why can’t you use animals that are less sentient?

Mice are the most commonly used animals for study of the somatosensory nervous system, because there is vast prior knowledge on their physiology. This foundation of knowledge has accelerated new advancements in the field. In most tested instances, the peripheral nervous system of mouse and man have been found to be highly similar and this is particularly true in the context of nerve injury and pain. Less sentient organisms e.g. fruit fly, can detect sensory stimuli and are amenable to genetic manipulation. However, their nervous system architecture and physiology is very different to that of mammals and is not suitable to address the questions of complex neural processing posed in this project. We will perform some studies under terminal anaethesia to investigate the cellular changes that occur upon certain stimulation. However, this only provides an indirect

measure of the sensory experience and so behavioural studies on awake animals are also required.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will follow robust standard operating procedures for handling, monitoring and general care of animals. For example, prior to behavioural studies animals will be habituated to researchers to reduce stress and anxiety levels during handling and testing. All staff members involved in animal studies will have substantial experience in husbandry or will be trained to such an extent prior to undertaking any work in this project. Strict monitoring of animals will occur, especially in cases of experimental manipulation which have the potential to alter animal behaviour. Where appropriate (e.g. before, during or after surgery) preventative measures (e.g. anaesthesia, fluid replacement, pain relief and heated cages) will be used.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will perform all studies in line with published (NC3Rs) and local guidelines. In particular we will adhere to the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines) on the reporting of scientific studies involving animals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will perform all studies in line with guidance found on the NC3Rs website and in the published literature. We will also monitor our local 3Rs webpage and attend 3Rs days run by our institution.

Home Office

NON-TECHNICAL SUMMARY

97. Investigating the role of cellular stress in neurodegenerative diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project will examine the impact of stress at a cellular level on the development of neurodegenerative diseases such as Alzheimer's disease and motor neurone disease. It will help us to understand how environmental factors that lead to cell stress might be involved in the development of these disorders.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Neurodegenerative disorders are a large and growing financial and emotional burden on society, with as yet only limited palliative treatments. Understanding more about how these diseases progress, and how different factors such as cellular stress may be involved will provide us with crucial insight into the disease mechanisms, and hopefully yield new novel targets for the development of treatments.

What outputs do you think you will see at the end of this project?

Outputs will include new information regarding how cells in the brain respond to acute stress such as fever, how this response is affected by aging, and whether this response might be involved in the progression of diseases such as Alzheimers and motor neurone disease. It will also aim to validate the use of specialised slice cultures as an alternative to live animal models for this kind of research. It will lead to several publications that will advance our understanding of how cell stress may be linked to disease, and help us understand more about the mechanisms that underly this interaction.

Who or what will benefit from these outputs, and how?

Initial benefits (obtained throughout the course of the project), will be seen by other researchers in the field of neurodegenerative diseases, as we will provide information to improve our understanding of what may go wrong as we age to lead to these diseases. We will also validate a model as an alternative to live animal research, which will be of benefit to researchers, and hopefully lead to a reduction in total animals used in this area of research. Longer term (over the course of the project and beyond), data obtained from this project may provide us with new targets to try to develop therapeutics to treat neurodegenerative diseases such as Alzheimers and motor neurone disease.

How will you look to maximise the outputs of this work?

All data obtained in this work will be widely shared throughout the academic community, with the goal of publishing all findings, positive or negative, in relevant scientific journals. As new findings are obtained, collaborations with both local colleagues and those further afield will be sought out to further develop our research. In addition, efforts will be made to engage with the local lay community to share any findings of interest, and in cases of specific interest to patient groups, we will liaise with the appropriate support group to try to disseminate the relevant information widely and accurately. **Species and numbers of animals expected to be used**

- Mice: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will work with adult mice as we are interested in how changes in our environment that result in stress to our cells, can impact on the function of proteins that are known to be important in diseases such as Alzheimer's and motor neurone disease. Since these diseases are known to be related to aging, we are also interested in whether the effects of this cell stress are changed as we age, hence some mice will be aged. The mouse has been selected as they possess a brain region called the cerebral cortex, which is only found in mammals, and this region is preferentially affected in many neurodegenerative diseases. Some mice in this project will have genetic alterations to allow us to understand more about the human version of a disease linked protein, or to more easily be able to identify and track the protein of interest in our studies. Understanding the cell mechanisms that may underlie an altered risk of developing a disease such as Alzheimer's may help us to develop new drugs to treat the disease.

Typically, what will be done to an animal used in your project?

The work planned in this project falls into three categories:

1. Normal and genetically modified mice will be exposed to a single short (2h max) period of increased temperature (42^{o}C max) designed to raise their core body temperature. They will be left to recover for a maximum of 48 hours before they are culled for tissue collection.

2. Normal and genetically modified mice will be exposed to a maximum of 6 short (2h max) periods of increased temperature (42^{o}C max) throughout their life-time. They will be aged out to a maximum of 2 years old, and during their life around half of these animals will undergo behavioural tests to look at how well their brain works. These tests will include tests of memory, and tests for mobility. In most cases, mice will be tested 4 times in their lifetime.

3. Neonatal normal and genetically modified mice will be culled prior to weaning to provide tissue for further study.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals undergoing a heat exposure may experience increased lassitude and discomfort during the procedure, however, this is expected to dissipate rapidly once they are returned to normal room temperature, and all animals are expected to return to normal within 30 minutes, with no long term consequences of either a single or repeated exposure.
Animals on this project that are maintained out to old age may develop various age-related issues that are common to mice, such as benign tumours or hair loss, and in some cases sore patches on the skin. If these effects are only mild, and cause minimal distress, the animal may be maintained for several months, however, if any of these issues are thought to cause significant pain, discomfort or distress, the animal will be culled.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The genetic modifications used in this project are not expected to have more than a very mild impact on the

general health and welfare of the animals. The acute effects of heat exposure are expected to result in moderate but transient discomfort to the mouse, hence all animals undergoing this heat exposure (approximately 50% of the experimental animals to be used on this project) would be classified as falling into the moderate severity category. All other animals are not anticipated to exceed a mild severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project is interested in the impact of cellular stress on brain function, and whether it might interact with the aging process to alter our risk of developing diseases such as Alzheimer's and motor neurone disease. One part of this project is designed specifically to understand how the cell stress response might function in the brain of a live animal, to improve our knowledge of this process, and also to explore whether we can successfully model this response using non-animal models. Another part of this project is interested in the long term consequences of a cell stress response on the function and dysfunction of proteins known to be important in neurodegenerative diseases, and how it might be altered as we age. This requires us to be able to study the brain throughout aging. Both the complex circuitry of the brain and the process of aging are challenging to model outside of a living animal, hence using animals is the only way to address these questions. Finally, we are also interested in understanding how any brain changes we see may relate to changes in behaviours such as learning, and these characteristics can only be measured in a living animal.

Which non-animal alternatives did you consider for use in this project?

Various cells, including stem cells (grown in a dish).
Brain slice cultures (thin slices of brain that are grown in a dish).

Why were they not suitable?

A part of this project is designed to try to validate cell or slice culture alternatives as a suitable model system to address some questions posed in this project, and where possible, these cultures will be used in place of live animals. Basic cell and stem cell cultures can provide information regarding what happens within a single cell, while brain slice cultures can provide information about local cell networks. However, neither of these options can fully mimic the impact of interactions between the millions of cells within the brain, and also how communication with, and the function of other parts of the body can impact on the brain. In addition, neither cells nor brain slices can give us any information on the practical behavioural consequences of any changes we see.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

Numbers to be used on this project have been estimated based on total numbers planned for the various different experiments to be conducted under the remit of this licence, as well as the number of animals likely to be required to maintain different lines of genetically modified mice. Initial studies have been designed to use the minimum number of animals assessed as being required to reveal any significant changes in response to the experimental protocol. For the longer term studies, numbers have been selected based on assessment of similar types of measurements in previous studies, and aim to be the minimum number of animals needed to identify whether a statistically relevant difference exists between the different experimental groups.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

During the design phase of each experiment, appropriate searches were done on each mouse line of interest to ensure we had access to all relevant information regarding expected outcomes in these animals. This was combined with more general knowledge on how much variation there is between control animals for each of the factors being measured. Extra consideration was made for a number of variables we control, including sex, age and background strain. The statistical approaches to be used at the end of the study formed a major part of the experimental design process, ensuring that all experimental approaches are robust.
The experimental design was also discussed with other researchers familiar with these kinds of studies, to further validate the design, and to ensure that we had included adequate control groups to each experiment, such that all data obtained will be valid.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

As far as possible, all experiments will be conducted longitudinally, to allow us to maximise the data obtained from each animal. For tissue harvest, all organs of interest will be harvested from each animal, and experiments will be designed to allow multiple follow ups in the tissue from each animal, thus minimising the total number of animals required.
Where relevant, small scale pilot studies will be conducted prior to each full scale study, with follow up analyses to ensure both that the objective is a valid one, and also that experimental design can be optimised to use the minimal number of required animals. This will ensure that unnecessary large scale experiments do not take place.
As far as possible, breeding strategies will be designed so that all animals from a mating are used for an experiment, and for general maintenance of a line, animal breeding will be monitored and controlled to ensure that we obtain sufficient mice to maintain the line, while minimizing the birth of mice that are not required.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain

management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

All the work planned in this project will use mice. These include healthy control animals and genetically modified mice that contain human and or tagged versions of proteins linked to neurodegenerative diseases. These genetic modifications are not anticipated to result in any significant distress to the mice, as the proteins will be the normal, healthy proteins. Rarely, the expression of the human version of the protein, or increased levels of total amounts of the protein, might result in very mild clinical symptoms as the mouse reaches old age, but this is anticipated to be very mild, and will not impact on the ability of the mouse to feed, drink and move freely about their cage.
To induce the cell stress that is central to this project proposal, the majority of live mice used in this protocol will be exposed to a chamber heated to 42^{O}C for a maximum of 2h, and in some cases, this may be repeated up to 6 times in their lifetime. This is expected to result in transient discomfort to the animal during the procedure, but will not cause any pain or significant suffering, and is not expected result in any lasting harm to the mice.
Where possible, tissue will be harvested from neonatal animals for follow up study, rather than conducting the work in live animals. The use of this model will ensure that animals do not experience any distress or suffering as a result of the experimental aims.

Why can’t you use animals that are less sentient?

This study is interested in how cellular stress impacts on the functioning of the brain throughout aging, and specifically how it interacts with proteins linked to neurodegenerative diseases, and how this interaction might affect disease relevant processes. Where possible, embryonic or newly born animals will be used to generate specific cell or brain cultures for investigation. However, the specific impact of cell stress to brain cells in vivo, either acutely or through aging over the longer term, can only be measured by exposing live animals to a cell stress induction. Since the brain region of specific interest to most neurodegenerative diseases is the cerebral cortex, only mammalian species which contain this can be used for this work.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The core body temperature of animals experiencing hyperthermia will be frequently monitored throughout the heat exposure and during the recovery period, and compared to baseline readings obtained prior to the procedure. Animals will only be left unattended once they have returned to their standard temperature. If the temperature or duration is found to cause unexpected distress, it will be immediately terminated, and future experiments modified to minimise such welfare costs.
Any animals undergoing frequent handling as a part of the experiment will be handled regularly prior to the start of the experiment, to ensure that this process does not induce unnecessary anxiety or stress to the mouse. In addition, all mice will receive appropriate training for all behavioural tasks prior to the onset of experiments, both to minimise anxiety and stress, and also to improve outcomes, which should ensure consistent data from all mice, and hence allow the use of the smallest number of tests and animals.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All work will be conducted following the general principals of the ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

I will regularly check the NC3Rs website for updates, as well as liaising with both our NTCOs and our local NC3Rs regional programme manager. Any relevant changes will be implemented immediately for all new experiments, and consideration will also be given to implementing changes to ongoing experiments, provided any such change is not expected to have an impact on the animals such that it may alter research outcomes.

Home Office

NON-TECHNICAL SUMMARY

98. Investigating the role of physical cues in nervous system function in wild type and transgenic animals

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants.

Key words

CNS development, neural disorders, mechanotransduction, mechanobiology, atomic force microscopy

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our brain is the most complex organ in our body. How nerve cells know where to send their long extensions called 'axons' to connect to distant cells is currently still poorly understood. To investigate how the growth of nerve cells is controlled during embryonic development and after injury, we combine approaches from biophysics and engineering with state-of-the-art cell and molecular biology. We are mainly interested in how the local stiffness of the surrounding tissue regulates neuronal behaviour, and how nerve cells sense the stiffness of their environment. The long-term goal of this research is to discover new mechanisms controlling the growth of nerve cells. This is important for fundamental research and might help us to understand how the brain develops. It might also help to develop novel strategies to promote neuronal regeneration after, for example, spinal cord injuries.
The questions that we want to answer within the lifetime of this project licence are:

- What are the mechanical properties of tissue and cells in health and disease?
- What is the role of physical cues in shaping the development of the nervous system?
- Do physical forces affect the behaviour of single cells?
- Does the stiffness of the surrounding environment affect intracellular mechanisms that direct cellular functions?
- Do different pathological processes affect neural functions through changes in the physical properties of their surrounding tissue?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

During the development of the nervous system, the nerve cells build long extensions (axons) which connect different areas in the brain. Only if the correct areas are connected with one another will the brain work properly. Previous work has shown that axon growth is strongly influenced by the mechanical properties of the surrounding tissue. We now aim to understand how tissue stiffness regulates neuronal function. By answering the questions above, we will contribute to a better understanding of brain development and neuronal growth. Our fundamental research may also lead to break-throughs in our understanding of different pathological processes in the nervous system such as, for example, neurodegenerative diseases or spinal cord injuries, which currently bind patients to wheelchairs for the rest of their lives.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

4760 mice
1150 rats
2650 Xenopus (frog)

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will need to breed and maintain genetically modified (mice, Xenopus) and non-modified animals (rats and mice, Xenopus). These protocols are expected to have minimal or no impact on the health and welfare of the animals.
In exceptional cases, a moderate impact on their health and welfare might be reached due to the nature of the genetic alteration. Specifically, in the wobbler mice used to investigate the impact of tissue stiffness on the onset and progression of a neurodegenerative disease, the degeneration of nerve cells may result in symptoms which strongly resemble amyotrophic lateral sclerosis (ALS). Clinical signs include reduced bodyweight, unsteady, wobbly gait, and reduced muscle strength of the head, neck and forelimbs. These animals will be closely monitored and humanely killed when weight loss reaches or is likely to exceed 15% of their initial weight. Furthermore, wobbler mice will be regularly assessed for both paw condition and gait impairment and be humanely killed as soon as either reaches a predefined severity level or if the mobility of more than two limbs is affected. Occasionally, wobbler mice may show eye infections due to grooming problems (< 5%). If the infection persists for more than three days despite treatment, the animal will be killed. Very rarely, wobbler mice may have seizures or show signs of laboured breathing (< 1%). These animals will be killed immediately.
We will induce superovulation in female frogs and mice. This means that we will administer a specific drug to females, which induces a stronger production of egg-cells and therefore a larger number of offspring. Minimal or no impact on the health and welfare of the animals is expected.
In order to breed some of the genetically altered mouse strains, we will need to order embryos which will be implanted into pseudo-pregnant mothers which will eventually give birth to the offspring, which is later used for breeding and maintaining the rest of the mouse colony. Since one method used requires a surgery this can affect the health and welfare of the animal. However we minimise this by using good technique and pain relief after surgery.
In order to induce a pseudo-pregnancy in a female mouse, we need to sterilize male mice that will be held with the females. This is essential because the female hormonal system changes in the presence of the male, resulting in the acceptance of the embryos in the female. Since the sterilisation method used requires a surgery this can affect the health and welfare of the animal. However we minimise this by using good technique and pain relief after surgery.
We will use genetically altered mice as well as genetically normal (wild type) rats and mice on this licence. Where it is necessary to use immature cells, pregnant dams will be killed after they have been anaesthetised before the pups are removed from the uterus and killed for use in our experiments.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Our work is set up to minimise the number of experiments with living animals whenever possible. We will make extensive use of cell culture when observations in the living animals will not be crucial. Unfortunately, we cannot replace our animal work completely with cell cultures (i.e. cells that are grown in dishes in the laboratory), due to the lack of available cell lines that are relevant to neuroscience studies. Our studies in living animals and in cell cultures will be accompanied by in silico models (i.e.
computer-based studies and simulations that do not require animal use), which will replace some experiments, and help us make informative predictions to only pursue promising experimental conditions.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Experiments will first be run as a pilot study with a minimum number of animals, taking into consideration good experimental design principles, including randomisation (random allocation of animals or tissues for experiments) and blinding (keeping researchers unaware of the experimental treatments until the study is over) to prevent potential bias of researchers and increase confidence in the conclusions of our studies. Successful pilot studies are the basis for standardising the protocol which reduces variability and mistakes, by using biostatistics approaches and principles of good experimental design. Importantly, in all our experiments we try to retain tissues for later use, and to share tissues among researchers whenever possible. Finally, in the context of frog experiments, our researchers arrange their experiments on the same days to minimise the numbers of frogs undergoing procedures as frogs lay thousands of eggs at a time.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We will always use the animal species of the lowest possible complexity. Wherever possible, we will work with frogs, an excellent animal model for studying the development of the nervous system, especially in the context of the optic pathway. The Xenopus optic pathway, where neurons from the retina grow along the brain to connect with the optic tectum (the analogous of the visual cortex in humans), is one of the best understood systems in the field of axon growth and guidance. Hence, much knowledge exists already, minimizing the number of required experiments. Then, we will test whether the findings which result from the frog embryo experiments can be reproduced in higher animals, we use rodents because their neurobiology is similar to humans'.
Mice and Rats will be housed in groups whenever possible. Environmental enrichment material will be provided (chew sticks and play tunnels). In order to reduce stress, non-tail capturing methods are used (cupping/picking-up the animals using a tunnel). Wobbler mice will always be housed with at least one non-wobbler littermate who can help them groom. They will be housed on soft bedding. To facilitate feeding, extra-long nipples will be provided on the water bottles and they will be served pellets from the floor.
The frogs are kept in a temperature-controlled aquatic environment. They are handled as little as possible as this induces stress in the animals. Instead of invasive methods for identification of the frogs, pictures are taken of each frog and used for identification to reduce handling-induced stress as well as inflammation and injuries. The frogs are provided with enrichment tunnels for refuge so they can display natural behaviours. The dedicated Named Animal Care & Welfare Officer (NACWO) attends and contributes to National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) events, and has provided a significant welfare improvement using visual welfare methods of diseases and clinical signs in photographs. Breeding and maintenance are accomplished with standard husbandry practices. Pilot studies are run on a few animals at a time and discussed on a case by case basis.
Surgical procedures will be carried according to the LASA "Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017)".
Furthermore, we will plan our experiments, maintain our animals, and monitor the components of our study according to the PREPARE guidelines.
We consider and apply ARRIVE guidelines for improving the reporting of our experiments. This allows us to maximise the published information and can therefore help to reduce unnecessary studies.
Everyone working with animals will be supervised will be supervised and trained by experienced members of the lab, and when people with the required expertise are not available internally, we will ask our animal facilities or our collaborators for training and support, either directly by their staff, or by providing the appropriate contacts

(e.g. using Training and competency databases).

Home Office

NON-TECHNICAL SUMMARY

99. Investigating the role of the extracellular matrix in disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	neonate, adult, pregnant, juvenile, embryo, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our previous work has identified that a genetic defect (a mutation) in a protein called collagen affects many tissues in the body and causes stroke, blindness and kidney disease for which there is no treatment. This project will investigate how these mutations in collagen cause disease, and we will determine if we can interfere with the mechanisms of these mutations to inform on possible new treatments.
A retrospective assessment of these aims will be due by 27 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Disease such as stroke, heart, eye and kidney disease are a major burden on Society and for many of there is an urgent need for better treatments. We have previously identified that mutations in collagen can lead to genetic forms of stroke, eye and kidney disease. By determining the mechanisms by which collagen causes these diseases, we will significantly increase our understanding of how these diseases develop. As these mechanisms may represent therapeutic targets, this will also guide the development of new and more effective treatments.

What outputs do you think you will see at the end of this project?

Outputs from this project will include the generation and publication of scientific manuscripts. In addition, oral and poster presentations will be generated and presented at local, national and international meetings.

A major output will be the increase in knowledge of how collagen plays a role in disease. Collagen has been implicated a large variety of diseases, many for which there are no treatments. Increasing knowledge gained in this project will help other researchers and industry in their research investigating these disease and/or other types of collagen.
Finally, this project will also help the workforce in the UK by providing high quality training to PostDoctoral Researchers and PhD students in multiple disciplines.

Who or what will benefit from these outputs, and how?

Impacts from this research will benefit directly other researchers, as well more indirectly and in the longer term will inform the general public and clinicians.
Researchers will benefit through publication of manuscripts and presentation during the course of this project. This also applies to clinical researchers. In the longer term the information on potential treatment targets for vascular disease may impact on patient management and ultimately treatment. In so doing this would be a benefit for the general public and the NHS.

How will you look to maximise the outputs of this work?

Outputs will be maximised through publications, oral presentations and public engagement activities including

press releases. In addition attending and presenting at scientific conferences will be used to disseminate information gained.
I am part of several multi-centre programmes of work with international collaborators. This will maximise the global reach of this work.

Species and numbers of animals expected to be used

- Mice: 3400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project focuses on the mechanisms of disease due to Col4a1 mutations with the aim of identifying therapeutic approaches. In addition identified mechanisms and treatment will also be investigated other vascular disease models for example Ehlers Danlos Syndrome due to Col3a1 mutations.
The animal models used are well established models for these diseases. For example, the identification of the human disease caused by collagen IV mutation was based on the analysis of mouse models with mutations in the same gene. This illustrates that mice are a very accurate model of the human disease and a powerful model to investigate how the disease develops and to analyse potential treatment strategies. In addition there is a wealth of genetic tools available to researchers and protocols are well established and standardised.
The mice models have been previously generated and recreation of other animal models such as zebrafish can be more difficult due to species differences: the cardiovascular system of mice has a much higher homology than the zebrafish cardiovascular system to the human cardiovascular system.
The use of well-developed protocols and pilot studies minimises the welfare costs to the animals. All possible measures will be undertaken to minimise animal stress as it is well established that stress has a strong influence on cardiovascular parameters such as blood pressure.

Typically, what will be done to an animal used in your project?

The vast majority of animals will be used for breeding and maintenance and analysis will be performed on tissues collected post-mortem. A minority of animals will undergo in vivo phenotyping protocols such as measurement of blood pressure and magnetic resonance imaging (MRI) to analyse brain haemorrhaging. The phenotypes of animals may be altered through administration of substances or altered diets with the aim of ameliorating the disease and identifying its underlying pathways.

What are the expected impacts and/or adverse effects for the animals during your project?

The adverse effects to the animals include the development of eye, kidney and vascular disease due to the mutation. Other very rare adverse effects are associated with the use of anaesthesia and protocols have been optimised to minimise adverse effects such as correct dosing and close monitoring of animals. As we will investigate older animals of more than 12 months old, we expect these to develop signs associated with normal ageing. In addition some of the adverse effects of the mutant animals can also increase with age. Close health monitoring of these animals will be performed to control the adverse effects.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect that the animal models (>95%) will have a mild severity limit, and <5% will have a moderate severity.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 27 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Currently no tissue culture and/or in vitro systems are available that accurately model the development of diseases that affect multiple tissues phenotypes, or their progression with increasing age.
Consequently, animal-based approaches are required to investigate the effect of mutations in genes and proteins on whole body biology or increase our knowledge of how diseases develop. In a similar manner to determine how a disease develops and whether a novel therapeutic strategy is able to modulate the disease, animal work is required.

Which non-animal alternatives did you consider for use in this project?

Whenever possible, we will employ in vitro approaches including cell culture to perform the molecular analysis of the mutations and how they affect how cells function. These cell culture experiments will also be used to investigate the efficacy of a treatment strategy. Data obtained from these cell culture experiments will then guide the animal-based work.

Why were they not suitable?

No tissue culture and/or in vitro systems are available that accurately model the development of diseases that affect multiple tissues phenotypes, or their progression with increasing age, necessitating research using animal models.

A retrospective assessment of replacement will be due by 27 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our extensive experience of working with animals combined with published research has generated a large amount of data that was used to estimate the number of animals required. Much of this has been based on our previous published research using these model and the techniques employed within this project. This has enabled us to establish the size of appropriate animal cohorts. In addition our experience with, and detailed knowledge of the used animal models has enabled us to develop appropriate breeding schemes and identify the number if animals that need to be generated. This experience has resulted in a significant decrease in the numbers of animals used.

Statistical analysis combined with pilot experiments has also been used.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The molecular analysis of mutations will be performed where possible in cell culture experiments. In addition, we have performed large mutagenesis screen on invertebrates, rather than in mice, which has signficantly reduced the animals used in this project. Data from these approaches will then guide our animal based work.

Where possible experiments on animal cohorts will be coordinated so that experiments will begin simultaneously for the entire cohort to allow optimal use of animals and reduce the number of animals. Statistical advice is sought to optimise approaches to ensure that maximum information is gained from the lowest number of animals. In addition, pilot studies are performed to determine how many animals are needed for an experiment, reducing overall the number of animals. Animals will be randomised, both sexes will be used and researchers will be blinded to the genotype and treatment status. This ensures effective blinding to prevent unconscious bias.
All procedures will be undertaken in accordance with current guidelines as listed on NC3Rs (http://www.nc3rs.org.uk/our-resources) comply with current good practice including ARRIVE guidelines.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To reduce the number of animals required we will collect multiple tissues of each animal and coordination of animal studies within the laboratory will ensure optimal use of animals. Performing multiple phenotyping assays will reduce animal numbers and provide an accurate characterisation of co-occurring phenotypes within one animal.
A retrospective assessment of reduction will be due by 27 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project uses mice as animal models due to the available genetic tools and standardised phenotyping protocols. The mice models have been previously generated, model the human disease, and recreation of other animal models is difficult due to species differences.

Why can't you use animals that are less sentient?

Although zebrafish is extensively used in analysing the development of the cardiovascular system, rodents are still the standard species for adult phenotypes. Due to size difference it is much easier to analyse adult mice compared to zebrafish and the rodent cardiovascular system has a much higher homology than the zebrafish cardiovascular system to the human cardiovascular system.
Human disease due to mutations in collagen progress with age and age is a major risk factor for stroke in the general population with the risk of stroke most increasing significantly above the age of 55 . It is therefore necessary to recapitulate these age points in our experiments through the analysis of mature adult mice (3-12 month of age) and in some cases older mice to investigate the impact of increasing age on disease.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Several approaches are adopted to implement refinement.
This includes pilot studies to develop expertise, optimise protocols and determine endpoints before the actual analysis. Measures will be undertaken to minimise animal stress. This includes training; for example animals will be trained before measurement of blood pressure. In addition whenever possible grouped housing will be utilised and animals are monitored daily. Further intensive monitoring will be performed where appropriate including on older animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All procedures will be undertaken in accordance with current guidelines as listed on NC3Rs (http://www.nc3rs.org.uk/our-resources), this includes the ARRIVA guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our animal work is performed with guidance from the NVS and Home Office Inspectorate, and we will keep abreast about advances in the 3R via the NC3R's website.
A retrospective assessment of refinement will be due by 27 January 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

100. Investigation of influenza virus infections in animals as models for human disease.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Influenza, Virus, Infections, Pathogenesis, Vaccines

Animal types	Life stages
Ferrets	juvenile, adult
Pigs	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to improve animal and public health and welfare in relation to influenza virus infection. This will be achieved by improving our understanding of viral pathogenesis and dissemination and to develop intervention strategies applicable to viral transmission between animals and humans. Animal models of human disease will be employed to investigate these factors.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Animal Influenza viruses have the capacity to infect humans and so can have a Public Heath impact. Indeed, pandemic influenza is included in the UK National Risk register of Civil emergencies. This licence therefore provides the essential capability to safeguard the UK by enabling research into influenza viruses that have the potential to cause pandemics in humans. Scientific results are communicated to stakeholders who include academia, biopharmaceutical companies and government organisations in the UK, USA as well as internationally .

What outputs do you think you will see at the end of this project?

The Objectives for this licence will support scientific capability to investigate influenza viruses that are constantly evolving, necessitating continued monitoring and research.

Objective 1 will specifically provide key reagents and tools to characterize influenza virus antigens, assess the antigenic relatedness of different influenza virus strains and data will be supplied to inform the selection of candidate vaccine viruses (CVVs) by international organisations.

Objective 2 will underpin characterization of influenza infection and outcome (pathogenesis) parameters and transmission of virus infections in a susceptible animal species (pigs) as an animal model for humans. Disease intervention strategies will be assessed to inform epidemiological assessments and disease mitigation strategies. The studies will also include preclinical investigation of intervention strategies such as candidate vaccines and antiviral drugs.

Who or what will benefit from these outputs, and how?

Beneficiaries will include funders commissioning the research carried out e.g. funding agencies in the UK and EU, governments and biopharmaceutical companies conducting fundamental research. International organisations will also benefit from knowledge-sharing. Ultimately the general public and global influenza community will benefit from the enhanced capability to inform influenza risk and disease mitigation strategies worldwide.

How will you look to maximise the outputs of this work?

The outputs of this work can be maximised through participation in collaborative studies with external organisations. As detailed, the establishment already has representation on several organisations and also has joint-funded projects that are active or in negotiation. This allows co-ordination of research efforts and good use of animals. Research findings are presented at external meetings and published.

The establishment ensures dissemination of knowledge to stakeholders, in accordance with the UK Government data sharing policy and also within the remit for engagement with international organisations . In the case of notifiable disease or increased risk information is disseminated through expert reports and disease risk assessments.
The establishment ensures active engagement with stakeholders, for example through the organisation's evidence and policy teams.

Species and numbers of animals expected to be used

- Pigs: 250
- Ferrets: 50

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The pig and ferret species can be naturally infected with influenza viruses of relevance for human infection and are therefore biologically relevant hosts. In addition, influenza virus infection results in similar responses in the respiratory and immune systems in humans, pigs and ferrets. For these reasons, both pigs and ferrets are both considered valid animal model species for human influenza virus infection. Where possible, an alternative to live animals is sought, for example cell or organ culture. However, for certain scientific questions where complex virus-host interactions need to be studied, it is not possible to replace a live animal. For example vaccine efficacy investigations require study of the complex interactions between virus and host immune system.

Typically, what will be done to an animal used in your project?

Procedures will involve inoculation of influenza virus with or without prior immune stimulation (e.g. administration of immunological reagents such as vaccines or antigens). Substances will be delivered by injection or into the nasal cavity. The study outcomes will generally be followed by analysing longitudinal blood and nasal samples. Some studies to assess virus transmission (either directly or indirectly) will require co-housing of contact healthy animals with infected animals or introducing animals into a virus-contaminated environment. Study duration will depend on the study design and scientific question to be addressed. Generation of an immune response normally requires 4-10 weeks. Influenza virus infection is normally monitored for a duration of 5-14 days.

What are the expected impacts and/or adverse effects for the animals during your project?

The main potential adverse effects are the clinical signs resulting from influenza virus infection which are predicted to be similar or milder to infection in humans. Clinical score sheets are used to ensure humane endpoints are applied and thereby minimise suffering. Transient mild adverse effects may also be experienced as a result of restraint during sampling or inoculation. Anaesthesia will be applied if anaesthesia is not more severe than the procedure itself.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Ferrets

Mild 100%

Pigs
Mild 99%
Moderate 1%

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Only viruses of scientific importance will be selected for full assessment in animals. A complete biological system is frequently required to fully study the course of clinical disease and virus-host interactions during infection and particularly the immune response of the host. For example, the mechanisms of virus transmission from one animal to the next and disease interventions such as protective immunity from vaccination, cannot be studied in non-animal alternatives.

Which non-animal alternatives did you consider for use in this project?

In vitro and ex vivo methods will be developed and used where appropriate, for example: continuous cell lines and organ or tissue explant cultures.

Why were they not suitable?

These alternatives are suitable to address some, but not all influenza research questions. Alternatives to animals cannot be used, for example, to address questions such as virus-host interactions, transmission, mechanisms of disease induction by a virus (pathogenesis) or vaccine efficacy.
Modelling of risk pathways in agricultural settings e.g. in a pig herd and assessment of risk or mitigating approaches at the animal-human interface also requires live animals of the relevant livestock species and animals representing human models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken

to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers have been estimated based on previous research programmes and project licences.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The use of a statistically valid minimum number of animals per study will be determined based on expert advice from a professional Biostatistician. Animal studies will be designed in a consistent manner so that inter-study comparisons and data analysis can be performed.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Animal studies will be designed to maximise collection of biological materials and, where feasible, run in parallel. This will potentially reduce the number of control groups required and therefore increase the data output and research questions that can be addressed.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Ferrets represent a valid human model for influenza infection and pigs can serve either as a human model or as a natural livestock host species.

Why can’t you use animals that are less sentient?

In studies involving study of virus-host interactions, it is not possible to use less sentient animals as study requires use of the biologically and epidemiologically relevant host species with a complete immune repertoire.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The species chosen are valid animal models or are a biologically relevant host e.g. pigs are the agriculturally relevant host for swine influenza and also represent a valid model for humans. Pilot experiments are used to refine protocols for the study of viruses with unknown properties e.g. to establish dose, route and timeline of infection required for infection and transmission. The research team strive to continually improve clinical score systems and the refinement of the humane endpoint(s).
The Clinical Score sheets used in this licence reflect refinements made over the course of the preceding licence. Animal species have their own specific and disease-relevant clinical observation criteria and score sheets. No animal will be allowed to progress beyond the described humane end point using a 2-3 times daily monitoring

system. On site veterinary teams and animal welfare officers (NVS and NACWO) are integral to each study. Clinical signs serve as study endpoints when the scientific objective does not require progression of disease.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Best practice guidance and information is obtained from NC3Rs, IAT and the RSPCA. Publications and articles are also reviewed during the approval process prior to each individual study. Where specialist training is required, inter-institutional exchanges and training visits are organised. Our team follows the ARRIVE2 in reporting data from animal studies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The Establishment applies the Culture of Care in animal studies. The Establishment frequently attends or organizes external meetings on laboratory animal welfare e.g. NC3R, RSPCA and IAT meetings. Staff attending these meetings provide meeting feedback reports locally. In addition, the Establishment has a Species Group Care and Use Committee and all PILs and NACWOs are invited to attend meetings. Specialist topics are presented and refinements, such as environmental enrichment, are communicated and opportunities are used for implementation. In addition, specialist knowledge exchange is organised by field and lab exchanges with other organisations conducting influenza research.

Home Office

NON-TECHNICAL SUMMARY

101. Investigation of Normal and Aberrant Haematopoiesis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

blood cells, cancer, stem cells, treatment

Animal types	Life stages
Mice	adult, embryo, pregnant, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim is to

1. Understand the mechanisms within the body that control normal and abnormal blood formation. Abnormal blood formation can lead to such things as blood cancers like leukaemia.

2. Test ways that would lead to improved blood formation systems where these are not functioning correctly.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Many blood-related disorders, cancerous and non-cancerous, remain incurable or inadequately treated. Improving the outcome of patients with such disorders will require better understanding of how disease develops at the microscopic level and within the whole animal. For the latter, use of carefully chosen mouse 'models' (mice that have had their genes altered to mimic the human disease) have proven very useful and often essential for research into how diseases develop in humans.
Our aim is to maximise the knowledge we can obtain from animal models of diseases of the blood and blood-forming organs and use it for the purpose of testing and developing potential new treatment approaches as we have done in several occasions in the past.

What outputs do you think you will see at the end of this project?

1. Provide proof that the processes and mechanisms identified in the laboratory as responsible for abnormal blood production are also true in the context of a living organism

2. Provide proof that treatments identified as promising for abnormal blood production in the laboratory are also active in the context of a living organism and do not cause unacceptable harm.

For example, this will provide the required proof-of-principle for proceeding with early clinical trials in healthy volunteers and then patients with bone marrow failure syndrome such as Diamond-Blackfan anaemia.

3. Provide proof-of-principle that transferring certain types of immune cells from donors to recipients of stem cell transplants will ameliorate complications of the stem cell transplant and thus improve the survival of recipients. We will also provide proof-of-principle that certain types of immune cells are more effective and

less toxic than others for the treatment of blood cancers. All these experiments are required for regulatory authority approval before these potentially novel therapies reach patients in clinic.

4. Information from 1-3 might be used by academic or industry stakeholders to develop improved treatments for patients

5. All the above will be freely available in the public domain in scientific publications

Who or what will benefit from these outputs, and how?

1. Patients with blood disorders

a) Patients with bone marrow failure syndrome may benefit from discovery of new therapeutic targets tested and validated in in vivo models. Validation of therapeutic targets will be followed by pre-clinical testing of candidate small molecules that may be identified by in vitro screening methods and validated in vivo, in relevant models. It is realistic that we will be able to identify therapeutic targets during the life of this PPL and possibly have an initial candidate small molecules to test and validate beyond that period.

b) Patients with blood cancers may benefit from novel, effective immune cell subset -based immunotherapy that is less toxic than current options.

It is realistic to expect that by the end of the life of this PPL and given availability of funding, the output from the proposed animal work will guide early and inform early clinical trials for patients with different blood cancers.

2. Academic or industry stakeholders

Validation of therapeutic targets in 1a may prompt industry or academic chemical biology laboratories to develop or re-deploy existing small molecules for therapeutic purposes.

Output from 1b will certainly benefit other laboratories around the world that develop immunotherapies and currently is the case attract early investment for further pre-clinical and clinical development from the pharmaceutical industry.

How will you look to maximise the outputs of this work?

Outcomes from our work on animals, once published, will be freely available in the public domain in scientific publications.

The applicant has a strong track record in patient public involvement and engagement activities in which the importance of using regulated animal experimentation is discussed.

We will continue engaging patients and their families with different types of heritable bone marrow diseases through research day events organised by our Institution.

The applicant is a Consultant Haematologist and an active clinician treating patients with blood cancers and with experience in clinical research. Therefore, he is well placed to lead clinical development of the cancer immunotherapy programme.

Our laboratory is also working closely with leading clinicians who lead one the largest single centre clinical services for the diagnosis and treatment of patients with heritable bone marrow diseases. Therefore, any advances with translational potential will be swiftly explored in clinic.

Species and numbers of animals expected to be used

- Mice: 7,780

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

For decades, mice have been used to investigate how blood cells are made and how the immune system develops and functions. They have also been used to test treatments such as chemotherapy drugs and immune therapies against cancer before they were tested in humans.
Mice are less sentient than higher mammals but are still relevant to human biology, and in particular normal and aberrant haematopoiesis, i.e. the process in the bone marrow that is responsible for the formation of all blood cells types. Moreover, there is a considerable amount of information in the literature that can be used as baseline and mutated and transgenic strains are available.
Extensive literature also shows that murine haematopoiesis at the molecular and cellular level greatly overlaps with human haematopoiesis and many human blood diseases, e.g. thalassaemia have been modelled successfully in mice.
Currently, immunosuppressed mice are considered the most appropriate animals for testing efficacy of different types of cellular immunotherapies including against cancer and for prevention and treatment of complications of blood stem cell transplants used to treat patients with blood cancers.

We will be using mouse strains that are optimal for studying growth of human cancer and testing of different treatments including immune therapies.
We will use mostly adult animals although is some cases, when we want to generate mice with high levels of human blood cells, we will use neonates.

Typically, what will be done to an animal used in your project?

Animals might receive cancer forming cells which will grow under the skin or in different organs including the bone marrow, and may produce tumours.
Tumour bearing animals will be treated with anti-cancer substances or immune cells with ability to eradicate tumours
How the tumour grows and how different anti-cancer treatments affect the tumours will be determined using advanced scanning procedures such as bioluminescence, CT and MRI scans
Animals might be injected with human blood stem cells (special human cells which can develop into many different cell types) to develop blood that has human features. Such humanised animals allow us to better investigate the function of human bone marrow and blood forming processes.
Blood will be taken to assess levels of different molecules and cells of interest.
In some instances, to improve the growth of transferred blood cells or tumour cells, recipient animals might be given doses of radiation.

What are the expected impacts and/or adverse effects for the animals during your project?

Mice which have been exposed to radiation may experience symptoms ranging from transient diarrhoea to radiation sickness for 2-3 days.
They will be more prone to infection afterwards but are held in conditions which minimise the chance of this happening.
Some animals will develop tumours but most are unaffected and are humanely killed when the tumour starts to affect their general health. In general, subcutaneous tumours will be expected to reach the set specific and humane end-points with 4 weeks from tumour cell inoculation. The corresponding duration for systemic tumour would be more variable and will depend on the type and cell dose of the tumour; in general end pints may be

met between 4 and 12 weeks post tumour cell inoculation.
Animals may be subject to minor surgical procedures but these will be carried out under anaesthesia and animals will be carefully monitored during recovery, and kept warm. Painkillers and antibiotics will be administered if necessary following surgery.
Some animals will have blood taken which will result in short lived discomfort.
Imaging is carried out whilst animals are anaesthetised and should not cause discomfort.
All animals are regularly monitored and advice sought from animal care welfare officers or veterinary surgeons where necessary.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Around 80% of mice will experience tumour burden, mild irradiation, multiple imaging sessions, administration of test agents and removal of blood samples. Applying humane endpoints to tumours, careful choice of substances, limitations to blood sampling and allowing the animals sufficient time between imaging sessions will result in a likely severity of moderate. The remaining 20% of mice will undergo higher dose of irradiation and risk radiation sickness which will be carefully monitored and humane endpoints applied to comply with the moderate level of severity of the corresponding protocols.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Studies in the laboratory only give information on cells in isolation. Since survival and growth of normal and cancerous cells depends on their highly complex interactions with a variety of cells, for example in bone marrow and organs that cannot be reproduced in the laboratory, the systems have to be tested in the whole animal. The same is true for studies on the effects of genes and changes to genes.

Which non-animal alternatives did you consider for use in this project?

Almost always, laboratory experiments will be performed using normal blood-producing cells from healthy volunteers or tumour cell types obtained from patients with blood disorders. For example, we use laboratory experiments to work out the functions of special cells in human bone marrow, blood or (umbilical) cord blood. Other types of cell involved in blood production are also used to study the impact of changes to genes or to look at the effects of substances such as drugs, growth factors, hormones, antibodies, immune cells) on cell survival and growth.

Why were they not suitable?

Laboratory-based studies mentioned above provide limited information and do not accurately reflect what happens in the complex whole body system.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Based on our experience from the previous PPL and the projected growth of our research programme especially in the field of cancer immunotherapy.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In all experiments performed, UK Co-ordinating Committee on Cancer Research (UKCCCR) guidelines will be followed at all times. Individual animals will be traced by earmarking at the outset.
In most experiments, post-mortem tissue will be harvested at the end of experiments to perform invaluable laboratory analyses.
We will pay particular attention to the size of the groups, keeping size to a minimum while allowing statistically and biologically relevant results to be obtained. In consultation with the Departmental Medical Statistician and with reference to the NC3Rs' Experimental Design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) we will undertake a formal calculation approach to estimate the sample size which will ensure delivery of experimental endpoints whilst using the lowest number of animals possible. In addition, we will be guided by similar published experiments if they exist. To minimise animal use when new procedures are being introduced, pilot experiments will be undertaken which will influence the design of further experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Mouse colonies will continue to be closely monitored to avoid excess animals. As appropriate, we will plan effective mouse breeding strategies which can provide us with the required experimental mice and controls.
To minimise animal use when new procedures are being introduced, pilot experiments will be undertaken, thereby determining parameters that will influence the design of further experiments.
We will use real time imaging systems such as bioluminescence to track tumour status and immune response. This removes the need to kill mice at certain time-points, resulting in a substantial reduction of animal numbers required to meet experimental end-points.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are the only animal species used in the proposed programme of work. Mice may wild-type (as they occur in nature), modified to alter a particular gene, or without a particular immune cell or factor. There are large numbers of mutated and genetically modified strains showing normal and abnormal blood creation. Many publications indicate the relevance of mouse models to human biology and these types of blood diseases in particular.

We will use genetically modified models as required and also models with transplanted tissue or organs of both normal and malignant blood formation.
We will use different strains with depleted immune systems which have been shown to be good receivers of grafted cells (both normal and malignant). Whenever possible, tumour cells will be administered under the skin into the flank as this causes least distress to the mouse. This method produces tumours with poor blood supplies, so this model is of limited value for testing substances which move round the whole body. Additionally, grafted tumours do not generally show the same disease progression found associated with abnormal blood production, so we will also generate and use genetically modified animals showing abnormal blood production.
To mimic features of some diseases such as acute leukaemia, it may be necessary to inject normal or cancer cells into veins.
To establish maximum tolerated, effective dose levels of treatments of interest (e.g. drugs, cells), and to minimise adverse effect on animals, drug dose will be given to mice in increasing amounts.
We will monitor the condition of the animals in all procedures regularly according to the protocol used and as agreed with NACWOs and veterinary surgeons. If unexpected distress occurs, mice will be humanely killed under terminal anaesthesia.
We will periodically seek alternatives to the use of animal models as reported in the literature and in online web sites dedicated to the promotion of the 3Rs such as CAMARADES (https://www.nc3rs.org.uk/camarades-nc3rs-systematic-reviewfacility-syrf) and Norecopa (https://norecopa.no/alternatives)

Why can’t you use animals that are less sentient?

There are no less sentient animals which have haematopoeic systems similar to that of humans. Mice are the only animal species used in this programme of work, with both mutated and genetically modified strains readily available.
Mice are less sentient than higher mammals but are still relevant to human biology, particularly in our field of study. Moreover, there is a considerable amount of information in the literature that can be used as baselines.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have adopted imaging of live animals as a means to reduce the number of animals required in cancer immunotherapy protocols.
In addition our protocols call for frequent monitoring of animal welfare and include measures to prevent and effectively treat procedural complications or pain, and failing these to promptly implement HEP.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will perform our experimental work according to guidelines issued by the Laboratory of the Animal Science Association (https://www.lasa.co.uk/), NC3Rs (https://www.nc3rs.org.uk/) and the United Kingdom Co-ordinating Committee on Cancer Research guidelines for the welfare and use of animals in cancer research (PMID: 20502460).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through scientific literature reporting improved models and methodologies for approaching animal work.
For example, we will adopt a specific genetically modified strain which allows higher frequency and levels of human (blood producing) cell engraftment that the original strain.
We will also stay informed of new developments in the field of 3R by engaging with NC3Rs (for instance, we have adopted the recently published ARRIVE guidelines 2.0); we will interact with
AWERB and the institutional 3Rs manager to be informed of relevant education events, e.g., webinars.

Home Office

NON-TECHNICAL SUMMARY

102. Investigation of the multiple factors that contribute to opioid overdose

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Heroin, Fentanyl, Respiratory depression, Opioid tolerance, Benzodiazepines

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Rats	adult, aged, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To determine the mechanisms behind the respiratory depressant effects of opioid drugs such as heroin, prescription opioids and illicit fentanyls (analogues of fentanyl).
To determine how tolerance develops to the respiratory depressant, analgesic and euphoric effects of opioid drugs and investigate how these change in the presence of non-opioid drugs of abuse or during periods of abstinence.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Overdose deaths involving opioids are increasing world-wide. Death in opioid overdose is due primarily to respiratory depression. It is overly simplistic to think that such deaths result simply from the user injecting too much opioid drug. In most cases overdosing is due to other factors such as (i) interactions with other drugs taken at the same time, (ii) loss of tolerance due to periods of abstinence, (iii) the use of highly potent opioids, like fentanyl, that break through the tolerance induced by regular opioid use, (iv) newer illicit fentanyls affecting the respiratory system in novel ways and (v) fentanyl induced overdose being less susceptible to reversal by naloxone.
In order to reduce deaths caused by opioid overdose there is an urgent need to understand more fully the processes involved in opioid respiratory depression in order to be able to explain the dangers, provide public health warnings and develop new more effective ways to reverse an overdose before it becomes fatal.

What outputs do you think you will see at the end of this project?

Our main research outputs are scientific papers in high quality scientific journals. We also give invited talks at scientific and drug treatment conferences as well as to government agencies such as Public Health England.
Who or what will benefit from these outputs, and how?

This research will provide important information on the factors contributing to opioid overdose, especially why the fentanyls are so dangerous and how we could better treat fentanyl overdose and reduce deaths. By quickly publishing our findings while the project is still on going the results from our research will help to inform other scientists working on similar topics and influence the direction of their research. For example, it will stimulate research into which opioid antagonists would be best to use in the treatment of overdoses involving fentanyls. Rapid publication and informing government agencies of our findings will contribute to the development of new guidelines and advice for drug users and treatment agencies on how to prevent and treat opioid overdose. In the

longer term the outputs from this research will inform future drug policy and contribute to Government strategies in relation to the social and health problems associated with opioid drug use.

How will you look to maximise the outputs of this work?

We will publish our research in high quality, international scientific journals.

In addition, all important discoveries will be communicated in non-specialist language to drug treatment workers and the general public via our university web sites, by press releases and by our involvement in public engagement. This includes talking with those involved in harm reduction, those going through drug rehabilitation, giving public lectures and speaking in schools. Being more aware of the dangers of opioid overdose and how it can be prevented is important in the wider community.

Species and numbers of animals expected to be used

- Mice: 1000
- Rats: 270

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult mice or rats of both sexes will be used. The experiments are not designed to examine age-related differences rather they are designed to examine drug effects on the mature brain.
The neuropharmacological characteristics of the respiratory depressant and pain relieving effects of opioids have been extensively characterised in these rodent species and closely mirror those in humans. The anatomical distribution of opioid receptors in rodent brain is similar to that in human. Consequently, rodents are an ideal model in which to undertake the studies outlined.

Typically, what will be done to an animal used in your project?

60% of mice will undergo acute experiments in which respiration will be monitored before and after injection of a drug. In some experiments their antinociceptive threshold will be measured. To do this mice will be handheld during the tail immersion/tail flick test with minimum restraint to avoid stress, During the respiration monitoring they will be unrestrained, being freely able to walk around the recording chamber for periods up to 2 hours. Water, but not food (munching on food tablets interferes with respiration monitoring) will be available. At the end of the experiment mice will be killed.
40% of mice will undergo a surgical procedure to implant a prolonged drug delivery device under general anaesthesia. Following a period of prolonged drug delivery (up to 7 days) they will then either (i) have their respiration monitored before and after an injection of a drug for periods up to 2 hours or (ii) undergo a period of drug abstinence (from 1 day to 3 months). At the end of the period of abstinence they will again undergo a surgical procedure to implant a prolonged drug delivery device under general anaesthesia and respiratory parameters monitored by plethysmography before and after an injection of a drug for up to 7 days. At the end of the experiment mice will be killed.
45% of rats will undergo general anaesthesia without recovery.
55% of rats will undergo a surgical procedure under general anaesthesia to implant a prolonged drug delivery device, telemetric recording device or have an indwelling IV catheter implanted and then be allowed to recover. Thereafter they will then either (i) have their respiration and respiratory muscle electrical activity recorded by plethysmographic and telemetric recording before and after drug injection, (ii) be allowed to self-administer drugs daily for up to 2 h or (iii) undergo tolerance induction prior to tolerance assessment being performed by administering a dose of opioid and measuring respiration and respiratory muscle electrical activity or by resuming the drug self-administration protocol. At the end of the experiment rats will be killed.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals that have undergone surgery may suffer some post-surgical pain however, to reduce this adverse effect animals will be administering analgesic drugs during the surgical procedure and post-surgery as required (NSAIDS and opioids).
Animals withdrawn from a prolonged period of opioid drug administration may incur withdrawal symptoms including diarrhoea and a jumpy behaviour, 'wet dog shakes' for up to 5 days. We will allow withdrawal to develop slowly by letting the brain concentration fall naturally. We will not administer antagonist drugs to these animals as that would evoke a more severe withdrawal response.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice - 60% Mild; 40% Moderate
Rats - 45% Mild; 55% Moderate.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The design of our experiments has taken into account the 3Rs. Where appropriate we will continue to perform in silico Molecular Dynamics simulations of drug-receptor interactions and *in vitro* experiments on cell lines expressing recombinant and mutated receptors as these can be used to generate, test and refine initial hypotheses as well as for proof of principle studies and thus direct our future *in vivo* experiments. They may also highlight similarity in action between different opioid drugs allowing us to group drugs and select only one drug from each group for further study *in vivo*. However, such in silico and in vitro approaches cannot be used to study drug-induced respiratory depression, analgesia and reward. Nor can they mimic the adaptive changes that occur *in vivo* to prolonged drug exposure as they do not replicate changes that occur in neuronal networks controlling complex behaviours, the influences of hormonal changes or long-term changes in neuronal gene expression.

There are two main reasons why animals have to be used in the proposed experiments

1. Opioid-induced respiratory depression, antinociception and reward can only be studied in the intact animal as these are integrated behaviours that require functioning sensory input to the brain as well as reflex neuronal responses to elicit the behaviour.

2. Previous research on cellular tolerance to opioids has used cultured neuronal cell lines and mature brain neurones contained in brain slices (from animals sacrificed by a Schedule 1 approved procedure) that have been exposed to opioid drugs such as morphine *in vitro*. That work has provided a wealth of important information on the potential mechanisms of cellular tolerance to opioids but it does not demonstrate that these mechanisms have a significant role in opioid action and the development of tolerance in complex behaviours resulting from simultaneous drug actions at multiple sites in the brain.

Which non-animal alternatives did you consider for use in this project?

In silico modelling of drug-receptor interaction *in vitro* experiments on cell lines

expressing recombinant and mutated receptors **Why were they not suitable?**

These non-animal alternatives would not provide information on how a drug could modify complex whole animal behaviours such as respiration, analgesia, and reward.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have extensive experience in the type of experiment described in this application and have published our data in peer reviewed publications that ensure high quality of experimental design that complies with the ARRIVE Guidelines and require justification of group sizes. Thus, for any drug treatment the group size (number of animals) will be the minimum number required to observe a robust,
statistically significant effect given the amplitude of the response expected and the variability in response amplitude we have previously observed between animals in that type of experiment.
For each experiment the total number of animals is based on
- (group size x number of drugs to be tested x doses of drug)/estimated success rate
Note: for most experiments the estimated success rate = 1 but in a few instances (e.g. implantation of recording electrodes or installing indwelling iv cannulae) the success rate may be less than 1 but no less than 0.75.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have limited the number of drugs and number of doses of each drug that will be studied. This has been done on the assumption that from our in silico and in vitro work we will be able to group drugs of similar properties (e.g. way they bind to the receptor, intrinsic efficacy, lipid solubility, reversal by antagonists) and then select exemplars from each group for the in vivo experiments rather than study all possible drugs.
We will perform as few solvent and antagonist drug controls as sound scientific practice demands to exclude

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The design of our animal studies will be predicated upon the results from *in silico* and *in vitro* cellular assays

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will monitor drug-induced changes in respiratory parameters in awake, freely moving animals using plethysmography. Apart from the mild discomfort of drug injection this procedure does not cause pain or distress.
To measure changes in respiratory muscle electrical activity we will perform experiments on the *ex vivo* brainstem-lung preparation. This reduces the need for experiments on awake, freely moving animals in which the recording electrodes and telemetric devices have first to be implanted under general anaesthesia. There is a need however to ensure that the drug effects observed in the *ex vivo* preparation are also seen in the awake, freely moving animals but the number of such *in vivo* experiments will be kept to the absolute minimum as required to demonstrate scientific validity.
In the self-administration experiments drugs will be administered iv to freely moving animals via an indwelling cannula inserted under general anaesthesia. Access to drugs is limited by the experimental protocol to prevent the induction of dependence and thus associated withdrawal occurring between testing periods.
Analgesia testing will be performed using standard thermal techniques. To prevent pain, suffering and distress the stimuli will be mild with the animals having the ability to withdraw themselves from the stimulus at any time. Stimuli will be applied at appropriate, controlled intervals with limits of intensity and duration to ensure tissue is not damaged.
To induce drug tolerance, we will implant under general anaesthesia slow release devices that provide continuous drug delivery for up to 7 days. These devices remove the need to give repeated drug injections every day which may be stressful and avoid periods of drug withdrawal that may occur between repeated drug injections.
For all surgical procedures performed under general anaesthesia local anaesthetic agents will be injected into the area surrounding the wound and applied topically to the wound area at the end of the surgery. Following surgery analgesic drugs will be administered to alleviate any pain caused by tissue damage during the surgery.

Why can’t you use animals that are less sentient?

Rodents (mice or rats) are the least sentient mammalian species that are appropriate for the opioid induced respiratory depression, analgesia and reward work. The neuropharmacological characteristics of the respiratory depressant and antinociceptive effects of opioids have been extensively characterised in these rodent species and closely mirror that in humans. The anatomical distribution of opioid receptors in rodents is similar to that in man. Consequently, rodents are an ideal model in which to undertake these studies outlined.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Our institution has recently issued new advice for the handling of rodents and introduced improvements in the quality of their caging environment. We will continue to refine our procedures in light of any new advice that is issued.
Each of the proposed experimental procedures has been in use in our laboratory or that of one of our close collaborators at the same institution for the past 10 years. Over that time they have been refined extensively.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will adhere to the revised ARRIVE Guidelines 2019 (available at https://europepmc.org/article/ppr/ppr85606) and any updated editorials issued by relevant UK scientific societies (British Pharmacological Society, Physiological Society) and their journals (British Journal of Pharmacology, Journal of Physiology) as well as by grant funding agencies. Surgical procedures will be performed aseptically in line with LASA guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will
- attend the annual 3Rs conference organised by our institution

- monitor the updating of guidelines on animal experimentation as they are issued by relevant UK scientific societies and their journals, and by grant funding agencies.

Home Office

NON-TECHNICAL SUMMARY

103. Ischaemia Reperfusion Injury and Organ Transplantation

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Pigs	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to characterise the mechanisms and consequences of ischaemia reperfusion injury in solid organ transplantation and to develop new therapeutic strategies using targeted drugs or cells.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Organ transplantation is a life-saving treatment for end-stage organ failure, but there is a growing disparity between the number of patients in need of an organ transplant and the number of suitable donor organs available. In January 2020, there were 6183 people in the UK currently waiting for an organ transplant, an increase in more than 1% on the previous year. Conversely, the total number of patients whose lives were saved or improved by an organ transplant fell by 2% in the same year. The total number of transplants performed also fell, ranging from a 3% decline for pancreas transplants to 20% for lung or heart-lung transplants. This is in spite of a 2% increase in the referral rate of potential donors, as well as a 1% increase in the overall consent rate for organ donation.

Between March 2018 and 2019, 400 patients died while on the active waiting list. A further 777 were removed from the active waiting list, mostly as a result of their deteriorating health and subsequent ineligibility for transplant, and many of these patients would have died shortly afterwards.

When an organ is donated for transplantation, it can spend several hours outside the body, without any blood or oxygen supply, before it is transplanted into the recipient. This interruption and restoration of oxygenated blood to the organ at the time of transplantation is known as ischaemia-reperfusion (IR) injury. This results in damage and death to cells, and ultimately can result in the organ being unsuitable for transplantation.

This project aims to improve our understanding of the cellular mechanisms that underlie IR injury and test the safety and efficacy of promising novel therapeutic agents. New treatments that reduce cellular injury and organ dysfunction will help overcome the national and international organ shortage by increasing the availability of organs suitable for transplantation. Improved rates of organ transplantation would have an enormous societal impact by shortening waiting lists, improving the quality of life of patients, and reducing complications and death from organ failure.

What outputs do you think you will see at the end of this project?

This project will generate academic outputs, new treatment products, and the basis of clinical studies which will have health and economic implications.

So far, our understanding of how cells and organs sustain injury during transplantation is from results of experiments in cells grown in special dishes in the laboratory, and in mice. We anticipate that project will not only confirm that what we know is also true in pigs, which are much more like humans in terms of size and complexity but will also extend to discoveries in the precise steps underlying IR injury. If our hypotheses are correct, the findings will enable us to design and test targeted treatments, and perform experiments to measure whether injury levels can be reduced this way.

The results of these experiments will be published in peer-reviewed journals and presented at national and international conferences. The treatments that come out of the project may be new intellectual property. From our experiments, we hope to learn what the best ways to deliver the treatments are, and how often and how much of the treatments need to be given. Altogether, these will generate a blueprint for clinical studies, which will bring successful new treatments to patients.

Who or what will benefit from these outputs, and how?

Throughout the project, researchers both inside and outside the field of transplantation will benefit from the above outputs. IR injury occurs in many human diseases, including myocardial infarction (heart attack) and stroke. The processes that occur in mitochondria, cells and organs are applicable to all of these diseases. Because the cellular processes underlying injury in transplantation, heart attacks and strokes are similar, we anticipate that our findings, including new drug treatments developed for use in transplantation, could potentially also be of benefit in treating heart attacks and strokes. Importantly, our raw data, publications and presentations will disseminate new knowledge to other researchers, who can then build on it.

The surgical and scientific techniques used in this project are very specialised, and therefore limited to a few experts who are able to carry them out. As this project proceeds, more researchers will be trained in these specialist skills. They will go on to apply them in collaborative or new studies in the future, ensuring the dissemination of good experimental practice and model refinement.

In the longer term, future clinical trials of the new treatments will be based on the results of this project. These trials will ultimately deliver safe and effective new treatments to patients with end-stage organ failure and patients undergoing organ transplantation. The new treatments may also be applied to diseases such as myocardial infarction and stroke, so a wider range of patients may benefit.

How will you look to maximise the outputs of this work?

This project represents an extensive multi-disciplinary collaboration involving multiple institutions. The knowledge created will be disseminated through presentations at national and international meetings and publications, which will include both successful and unsuccessful strategies investigated in the study.

Species and numbers of animals expected to be used

- Pigs: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Pigs that weigh approximately 40–70kg have been chosen as they closely approximate the anatomy, size, physiology, and function of human organs. Techniques for organ retrieval, supplying blood to the organ outside the body (ex vivo perfusion), and transplantation are transferrable between pigs and humans, allowing us to closely model how organs are affected when their blood supply is interrupted (ischaemia) and when the blood supply is restored (reperfusion). This project will allow us to confirm that our mechanistic findings from mice are translatable to larger animals. Once the large animal (pig) model has been developed it will be used to test the safety and efficacy of our new treatments prior to the treatments being using in humans.

Typically, what will be done to an animal used in your project?

We will use commercially-sourced adult pigs for all of our experiments. When possible, animals will be trained to eat the substrate in which a sedative is administered before induction of terminal anaesthesia. Animals will undergo general anaesthesia, they may then be treated with therapeutic agents or cells, and finally multiple organs will be retrieved for the study of ischaemia reperfusion injury. The animals used in this project will be humanely killed at the end of surgery whilst still under anaesthetic (non-recovery). This means that any given animal will undergo anaesthesia and surgery once, and will not experience any pain, distress or suffering as a result of any procedure.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals will receive a general anaesthetic from the beginning of the experiment which will last to the end of the experiment after which they will be killed by the administration of an overdose of anaesthetic. The procedures do not involve reawakening the animal; therefore, no adverse effects are anticipated.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Pigs: 100% Non-Recovery. All animals will undergo non-recovery procedures, as described above.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Ischaemia reperfusion injury in solid organ transplantation is a complex biological process involving crosstalk between cells, organs, the immune system and more. There is a gap in applying the knowledge we have generated using basic experimental models of ischaemia reperfusion injury to clinical transplant practices. We have used these basic experimental models to identify new therapies, but before these can be offered to patients, it is essential that we carry out clinical trials to ensure that these new therapies are both effective and safe. This project will provide us with a more complete understanding of the underlying pathological processes, help us shortlist the most promising new therapies, and inform the design of clinical trials in humans.
Which non-animal alternatives did you consider for use in this project?

This project has been preceded and informed by experiments using cells grown in special dishes in the laboratory (*in vitro*) and donated human organs that have been declined for clinical transplantation. Non-animal alternatives have, therefore, been extensively used as far as possible, in order to replace animal experiments.

Why were they not suitable?

Use of cells does not enable the complexities of organ injury during transplantation to be assessed fully. While we have and continue to make extensive use of human organs, these are very rare and access to them is very unpredictable. Moreover, because some of the human organs are of less-than ideal quality (which is why they were not used for transplantation), they are not always able to be used to generate robust and reproducible data.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals per group have been estimated based on the preliminary data obtained from previous experiments, which we have used to estimate the variance in the data and the anticipated effect size in the treatment groups. We estimate a group size of 5 per group, with a total of 20 experimental/control groups, requiring a total number of approximately 100 animals. This is likely to be an overestimate, as in our experimental design, group sizes of 4 per group are anticipated to generate biologically and scientifically significant data. The overestimate is included to account for cases when it is not possible to generate data for technical reasons.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have designed the experiments to maximise the number of organs and tissues that can be obtained for experimental use from every animal. For example, the heart, liver and kidneys, or biopsies from these organs, can be obtained from the same animal, enabling us to study mechanisms of injury (and response to therapy) to multiple organs in the same animal. In experiments using kidneys, we plan to use one kidney from each animal as a control and the other kidney as the experimental organ, thus reducing inter-animal variability and increasing the reproducibly of the data. By obtaining tissues under terminal anaesthesia, we will be able to ensure that the blood pressure and heart rate of the animals are consistent. This will reduce inter-animal variability, a key source of biological noise which can reduce the ability to generate biologically and statistically significant data.

We will continue to use the NC3Rs Experimental design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) and the PREPARE guidelines (https://norecopa.no/prepare) for our ongoing experimental designs.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The proposed project builds on a wealth of pilot data, generated from small animal (mouse) models and use of human organs. We will therefore only pursue hypotheses and progress the use of therapeutic agents that have been shown to demonstrate promise in pilot studies. Moreover, we will actively share tissues from the animals with other researchers in order to reduce the number of animals used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The animals will be acclimatised to their new accommodation before the experiments, according to the guidance

of the Named Veterinary Surgeon at our establishment. Animals will receive a general anaesthetic from the beginning of the experiment which will last to the end of the experiment after which they will be killed by the administration of an overdose of anaesthetic. The animals will therefore not endure pain or suffering.

Why can’t you use animals that are less sentient?

Pigs are chosen for the proposed experiments because the size and anatomy of the organs closely resembles those of humans. The data generated from these experiments will therefore be directly applicable to humans and prime the design and conduct of human clinical trials. The research is using the least sentient model because all animals will be terminally anaesthetised in the experiments.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The Named Animal Care and Welfare Officer (NACWO) and animal technicians will be involved in sourcing animals, providing suitable acclimatisation and handling and environmental enrichment, all of which will improve animal experience. As housing and handling of the pigs, prior to the experiments, can contribute to contingent harm, we will monitor the pigs for any evidence of stress or adverse effects. If necessary, we will seek guidance and input from the Named Veterinary Surgeon (NVS) and explore measures to reduce such stress. For example, the acclimatisation period will be extended, or additional environmental enrichment will be provided as appropriate. Under the guidance of the NVS, palatable sedatives will be administered to reduce stress prior to terminal anaesthesia if necessary. During anaesthesia, the animals will be continuously monitored (heart rate, blood pressure, oxygenation and temperature) to ensure the depth of anaesthesia is appropriate.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The PREPARE guidelines and NC3Rs Experimental Design Assistant (EDA) have been used to design this project. These have aided in the comprehensive consideration of logistical, legal and ethical, quality control and experimental design and statistical analysis issues. Home Office Advice
Notes, Codes of Practice, and regulations for personal and project license holders will be adhered to. We use the Establishment's 3Rs search tool as well as the NC3Rs and other websites (e.g., www.thepigsite.com) websites to keep up to date with new techniques and refinements as they are published. We will also actively explore and adopt any experimental procedures and techniques reported by other groups that can help refine the conduct of the experiments.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I have been actively involved in the ethical review of other scientific projects at the establishment. I actively engage with and keep informed about advances and developments in the 3Rs, including publications, conferences, meetings and new guidelines.

Home Office

NON-TECHNICAL SUMMARY

104. Lipid regulation of cardiovascular disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Cardiac disease, clotting, high fat diet, specialised fat molecules,

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The primary aim of this work is to determine the role that newly discovered lipids (fats) called "eoxPL" play in cardiovascular disease and abdominal aortic aneurysm, specifically through their ability to modulate blood clotting. It has been recently found that these lipids are made in the blood vessels of mice with both disorders, and that mice that are not able to make these lipids are protected from disease. Studies have shown these lipids can alter different aspect of vascular disease by modulating both clotting and underlying vessel wall

inflammation. We now need to better understand the molecular mechanisms involved in order to identify new ways to prevent or treat these diseases.
A retrospective assessment of these aims will be due by 02 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Cardiovascular Disease CVD causes 26% of all deaths in the UK; which equates to over 150,000 deaths each year or one person every three minutes. Along with this, abdominal aortic aneurysm is a complex silent disease, often co-existing with CVD (such as aneurisms and atherosclerosis), that has no effective treatments current, and is of high mortality. These diseases represent a massive public health concern, as well as a considerable economic impact.

The newly discovered lipids generated by enzymes (called lipoxygenases) appear to play a major role in the progression of cardiovascular disease and aneurysm formation, but the specific ways in which these lipids act still needs further investigation. The potential benefits of this work could include new treatments to prevent cardiovascular disease or aneurysms, or identify blood proteins that could be used to identify “at risk individuals”. We may also discover new drugs that could be developed for treatment of bleeding disorders.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice (wildtype and genetically altered strains) will be used, and the total number will not be greater than 12,500 in total. Only a proportion (3,500) of these will undergo complex biological testing, the rest will be for breeding purposes

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Of the 3,500 mice that undergo biological testing only a very small proportion, less than 35, may die as a result of complications that result from procedures.

1) Sudden death caused related to Ang II aneurisms.
For one of the knockout mice (or double knockouts) treated with an aneurism agent (Ang II), there is a risk of sudden cardiac death. Daily checks are conducted on the mice that are receiving Ang II, and mice showing adverse effects will be humanely killed. In addition, any mice found dead whilst under protocol 3 will be necropsied to evaluate whether an aneurism rupture has occurred

2) Breeding defects observed in knockout mice.
The knockout (ApoE) breeders show a reduced breeding pattern, compared to other knockouts and higher pre-weaning loss rate, for this reason animal care staff perform regular monitoring on mouse pups and license holders conduct weekly visual inspection on these mice.

3) Skin lesions induced by high fat diet in ApoE-/- mice
To assess this, daily health screening will be conducted on the mice. Should mice not respond to treatment over a course of 3 days then mice will be humanely euthanised.

4) Surgical procedure to implant minipump
Where surgical procedures are applied, additional appropriate control measures are in place for all adverse effects to ensure that all animals will be monitored closely, and appropriate action taken. All animals will be humanely killed if adverse effects cannot be treated.
A retrospective assessment of these predicted harms will be due by 02 March 2026

The PPL holder will be required to disclose:
- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement
State why you need to use animals and why you cannot use non-animal alternatives.

To study cardiovascular disease and aneurysm, the use of mice is unavoidable because this can only be studied properly in vivo.
Disease development involves the coordinated interaction of multiple cell types with the specific micro environment and physical stress/strain parameters which cannot be modelled faithfully with cell or computational models. We also extensively use non-sentient alternatives including cultured cells, human tissue obtained post-operatively from patients and healthy volunteer human blood.
A retrospective assessment of replacement will be due by 02 March 2026

The PPL holder will be required to disclose:
- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction
Explain how you will assure the use of minimum numbers of animals.

Based on previous work, appropriate group sizes that give a defined statistical power will be used. Results will be monitored as studies are undertaken to determine whether subsequent experiments could use fewer mice
Where possible, we will breed lines as homozygotes.
We will cryopreserve lines we are not actively using and provide tissues to both internal and external collaborators.
A retrospective assessment of reduction will be due by 02 March 2026

The PPL holder will be required to disclose:
- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We will minimise suffering by applying procedures which where possible are non-invasive, and only lead to short periods of minor discomfort and stress and ensure less than 1% of all mice experience more adverse effects such as aneurism related deaths. Further to minimise harm to the animals, animals will be monitored weekly and then daily (whilst undergoing procedures) and where there is any concern, advice will be sought from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer, and prompt appropriate action taken. Pain relief and appropriate anaesthesia will be used as standard for surgical techniques or when needed to reduce pain. Habituation will be used to acclimatize mice to restraint stress. A Treatment plan for skin lesions is in place so that as soon as mice present with symptoms treatment can be started immediately.
Mice represent an excellent system in which to study cardiovascular disease and aneurysm, since they possess many of the same genes as humans and also display similar disease progression. Mice are also an ideal species due to our ability to genetically and environmentally modify them.

Mice with a gene mutation (*ApoE* gene, a protein that is important in handling plasma lipids/fats) are susceptible to elevated blood lipids and cardiovascular disease. As such these mice represent a robust mouse model of cardiovascular disease that have been extensively characterised over the last 20 years. This again makes them an ideal model to study the pathophysiology of this disease.
A retrospective assessment of refinement will be due by 02 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

105.

Macrophages as a cell therapy for liver disease Edin 003

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Liver fibrosis, Cell therapy, Macrophages, Cirrhosis

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged
Rats	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to identify, develop, and test if a genetically-modified macrophage-based cell therapy candidate can serve as a treatment for acute and chronic liver injury. Currently, the prevalence of liver disease is increasing, and there are no effective therapies other than liver transplantation.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

If this project is successful, we will develop a genetically-modified macrophage for cell therapy for chronic liver disease, which would be the only treatment option for patients other than liver transplantation. The ultimate goal of this project is take a new cell therapy product into phase I clinical trials in patients with severe liver cirrhosis. Currently, there are no effective treatments for patients with cirrhosis of the liver, other than liver transplantation. Here, we hope to gain evidence that human macrophages, a type of white-blood cell, can serve as a cell therapy in animal models of liver fibrosis. We will genetically-engineer the human macrophages to vastly improve their anti-fibrotic potential by targeting certain genes, which we predict will make them very effective at treating liver disease. If successful, genetically-modified macrophages could then be the first approved therapy for chronic liver disease other than liver transplantation. A macrophage-based cell therapy could help improve liver function in patients with liver cirrhosis and prevent them needing a liver transplant.

What outputs do you think you will see at the end of this project?

The main outcomes of this project, if completed successfully, will be:

- a treatment for chronic, and possibly acute liver injury, to be further tested in patients in order to have it approved as a therapy for chronic liver injury by the relevant regulatory authorities

- patents to protect the intellectual property around new macrophage products generated during the duration of this project

- papers to ensure that the scientific community can scrutinise our results appropriately, and to ensure that our findings can be broadly utilised in basic research

- community and public engagement material to allow the general public to understand what cell therapy is and how it can be translated from the laboratory to the clinic

- abstracts to be presented at conferences in the form of posters or oral communication. This will: (i) ensure the professional development of early career scientists working with us; (ii) allow the establishment of new collaborations; (iii) disseminate the knowledge gathered thanks to the present project

Who or what will benefit from these outputs, and how?

Short term outcomes will be an increase in the public knowledge on cell therapy thanks to our regular involvement with public and community engagement projects. This is an ongoing commitment, with several events organised by our local public engagement office every year.
Mid-term goals will be: (i) the patenting of products that we may deem interesting to be brought forward for clinical assessment; (ii) the presentation of results around such products at conferences and meetings. We estimate that 2 years at least will be necessary prior to reaching these goals.
Long-term goals will be the approval for clinical trial of a macrophage product, and its testing in phase I/II trials. Further, we aim at publishing these results in well respected, peer reviewed international journals. We believe we can reach these goals during the last year of this project, and perhaps the year following the natural conclusion of the study.

How will you look to maximise the outputs of this work?

This project is the result of the collaboration between three research groups within the Company:

1. a **discovery** research group will identify and generate several genetically-engineered macrophage candidates

2. a macrophage **development** research group will pre-screen the cell candidates in vitro

3. an **in vivo pharmacology** team to test only best-in-class cell candidates in animal fibrosis models, thereby reducing the overall number of animals required

We will also look at maximising outreach of our data by presenting at conferences and publishing results (both positive and negative), thereby providing the scientific community as a whole with a blueprint to test this type of therapy using other cell types (e.g. cell therapy of liver disease using other cell types, e.g. hepatocytes).

Finally, we will ensure that our junior scientists are trained to design experiments that deliver reproducible and meaningful results; we will also ensure that they have received appropriate training to carry out complex procedures according to best standards, as set in the locally-approved standard operating procedures. This will maximise the chances of obtaining robust, reproducible and publishable results with the use of the minimum number of animals.

Species and numbers of animals expected to be used

- Mice: 6000
- Rats: 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Inbred rodents (i.e. mice and rats that are almost genetically identical to each other) are the best choice for this project because they low biological variation between individual animals. Consequently, this will reduce the natural variability we observe in our experiments and means we require fewer animals to faithfully evaluate our new cell candidates. In this project, we will test the safety and efficacy of human macrophages in animal models

of acute and chronic liver disease. In most cases, we need to use immunodeficient rodent strains (i.e. rats and mice that lack certain immune cells) to allow the engraftment of human cells. Models of immunodeficient rodents are widely commercially available and are usually on inbred background strains to keep biological variation low. We propose to use adult rodents, not younger than 8 weeks, as the pathology we are experimenting a therapy for appears in the adult life of patients. Finally, inbred rodent housing and husbandry is highly standardised, ensuring consistent and robust results.

Typically, what will be done to an animal used in your project?

We will model several types of liver disease in animals: chronic liver fibrosis, acute liver injury, and liver ischaemia. We also intend to understand the distribution of the cells in animals in healthy and diseased animals.

1. Acute liver injury will be modelled by a single injection of a substance that is toxic to the liver i.e. induced acute liver injury. Mostly, we use a high-dose of paracetamol in mice (acetaminophen, APAP) due to its clinical relevance. Typically, mice with liver injury will receive the macrophage cell therapy candidate (or vehicle alone) via intravenous injection (tail vein) usually 16 hours following paracetamol administration. Mice will be culled at various times after macrophage treatment to understand how safe and effective our new cell therapy is. Liver and blood harvested from the mouse will be analysed in the laboratory to determine the effect of macrophage therapy on liver injury, inflammation, and liver regeneration.

2. Chronic liver disease will be modelled by repetitive treatment with carbon tetrachloride (CCl_4) twice-a-week for up to 16 weeks, depending the degree of liver fibrosis we wish to induce in animals (usually 8-12 weeks is sufficient). During the final weeks of treatment, animals will receive either macrophage cell therapy or vehicle alone (saline, control mice) via intravenous injection once-a-week. Mice will be culled at various times after macrophage treatment (from 1 day - 1 month after cell therapy injection). Another way to induce chronic liver disease is to administer thioacetamide (TAA) dissolved in drinking water. TAA treatment also may vary in length (6 - 22 weeks) depending on the degree of liver fibrosis we wish to induce. The TAA model is particularly useful because the resulting fibrosis is stable (especially in rat) whereas fibrosis automatically resolves in other liver fibrosis models (e.g. CCl_4). Therefore, the TAA model will therefore allow us to test macrophage cell therapy after stopping TAA treatment, and follow up its safety and efficacy for a number of weeks after injection (up to 12 weeks). This model will also allow us to model the injection of multiple doses of macrophages after the cessation of TAA injection (e.g. 4 doses over 4 consecutive weeks).

3. Liver ischemia is a useful model to reflect the injury processes that occur during liver surgery (e.g. when resecting cancer) where blood flow needs to be temporarily arrested. The mechanisms of injury differ to acute and chronic disease models and this model allows an alternative injury in which to test our cell therapy. Liver ischemia can be modelled in mice using a surgical procedure by stopping blood flow to the liver by gently clamping certain vessels for a fixed period of time. Once the clamps are removed, mice could receive macrophage therapy candidates (or vehicle) at different times, and though different routes to find the most effective treatment combination.

4. A small group of animals with liver disease will be subjected to non-invasive imaging to study where the macrophages go in the body after injection. This is important to determine any side effects, e.g. due to migration of cells to an unwanted site. During imaging, the mice will be under general anaesthesia for a short period of time to immobilise them. Their bodies will be scanned and images of the various anatomical compartments will be visualised on a computer to track the injected cells in real time. These studies will help us understand where the cells go in the body, and how long the cells stay there for. Data from these studies will allow us to better design future experiments and to know how many and how often macrophage cell therapy candidates need to be dosed.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals with chronic liver disease can develop weight loss and show temporary signs of illness, which can include: loss of appetite, piloerection (striking coat), reduced mobility, reduced response to external stimuli and general malaise. Very rarely, animals can develop liver tumours (hepatocellular carcinoma), most likely on the TAA protocol. Normally, general malaise develops after the injection of the substance used to induce liver injury, and for a short period of time afterwards (no more than 2 hours). The other side effects may develop over time (from 3 weeks of treatment onwards) in a minority of the mice involved in the experiment. Weight loss is a good proxy measure, together with behavioural changes (grimacing, withdrawal and subdued behaviour), for the development of liver tumour and it will therefore be monitored closely.
Mice with acute liver injury can develop weight loss, hypothermia, piloerection, general malaise, grimacing and withdrawn behaviour, which usually only lasts for 12-16 hours after drug administration and then quickly subsides. Usually, mice fully recover within 48 hours and regain full health.
Potential side effects of macrophage injections are infections (usually only in animal strains which are immunocompromised), pulmonary embolism (clot in the lungs caused by the injected cells) and a risk of increased inflammation. Leading up to this project, we have so far injected more than 100 mice with chronic liver disease with various type of macrophages, and we have never observed any of these side effects, which demonstrates that injecting macrophages is safe. Mice are to be observed for 15 minutes post-injection and then once every 24 hours to monitor for side effects.
During live imaging, mice undergo general anaesthetic. Animals can experience breathing issues, and an overdose of anaesthesia could potentially be fatal. To avoid this scenario, breathing will be monitored constantly during imaging, and percentage of gaseous anaesthesia carefully adjusted accordingly.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In this procedure project licence, the maximum expected level of severity is moderate.
The proportion of animals that reach moderate severity is protocol-dependant summarised in Table 1 and Table 2 for mice and rats, respectively.

Protocol Number	Protocol Name	Proportion - mild severity	Proportion - moderate severity
1	Breeding and maintenance of genetically-altered animals	100 %	0 %
2	Induction and resolution of liver fibrosis	10 % (healthy group, vehicle control only)	90 % (i.e. all animals entering liver fibrosis protocols)
3	Induction and resolution of acute liver injury	20 % (healthy group, vehicle control only)	80 % (i.e. all animals entering acute liver injury protocols)
4	Dose-finding procedure	40 % (mostly pharmacological agents)	60 % (mostly toxicological agents)

5	Ischemia-reperfusion injury	0 %	100 both receive	(controls and treatment groups surgical procedure)

Table 1: Mouse severity classification an d proportionality

Protocol	Protocol Name	Proportion - mild severity	Number	Proportion - moderate severity
2	Induction and resolution of liver fibrosis	10 % (healthy group, vehicle-control only)		90 % (i.e. all animals entering fibrosis protocols)
4	Dose-finding procedure	40 % - mostly pharmacological agent dose-finding		60 % - mostly toxicological agent dose-finding

Table 2: Rat severity classification and proportionality

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

When planning the use of a cell therapy, the first concern is safety. Whilst some safety tests can be carried out in a dish (in vitro), several adverse events commonly observed when using biological therapies depend on a number of complex interactions that happen within a living organism. Reproducing faithfully these complex interactions *in vitro* is still not possible. Therefore, the use of rodent models is still essential to study efficacy and safety of new cell therapy.

Which non-animal alternatives did you consider for use in this project?

The first part of the discovery 'screening' phase is to choose and test genes to genetically-engineer in macrophages to generate our new cell therapy candidates. All candidates will be thoroughly tested using a battery of in vitro measurements first to ensure:

1. the genetic engineering has worked
2. the genetic engineering is optimal and can't be further improved

3. the new candidate shows promise as an effective treatment for liver fibrosis in culture

This strategy will ensure that only the cell therapy candidates that have the best chance to work are tested on mice, thereby reducing the number of mice needed to carry out the experiments.
Some non-animal alternative models exist, including collagen degradation assays, and may be utilised in the development testing phase, however critical limitations exist meaning these alternatives do not faithfully model the true biology that underpins liver fibrosis.

Why were they not suitable?

In culture, we can use individual assays to separately test different aspects of macrophage behaviour and function. These include the ability to:
- eat pathogens and/or dead cells (phagocytosis)

- migrate through a barrier (similar to the migration through the scar in the liver that the macrophages have to do once injected in a patient/mouse)

- actively reduce the scar (collagen) by breaking it down

- keep a stable behaviour in the presence of inflammatory molecules

- produce molecules that can help to switch off inflammation and promote regeneration of the liver

However, in a fibrotic liver, the macrophage is likely to perform all these functions at once. Therefore, a mouse model in which the macrophage used as cell therapy can behave in a fully physiological manner is required. Finally, previous studies suggest that macrophages interact with many other cells in the liver, including scar-deposing cells (activated hepatic stellate cells) and biliary cells. These complex interactions are crucial to fully understand the therapeutic effect of the macrophages during liver fibrosis. Unfortunately, we are not able to recapitulate this complexity in a dish, and therefore we need a mouse model to do so.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

We have used a combination of approaches to determine the number of mice to be utilised in this project:
1. based on preliminary data, we have applied appropriate statistical analyses to predict how many mice per experiment we would theoretically need

2. for each experiment we have considered what the best experimental design could be, depending on the biological question we wish to answer with that specific experiment

3. based on the available scientific literature, and our previous experiments, we have selected the best models to answer our biological questions

4. we have used freely available online tools to help with the experimental design, to ensure it is optimised and tailored to our needs

5. we have follow the current national guidelines to design experiments, and we have kept in mind the recommendations for best practice during data analysis when designing the experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In order to optimise the number of mice utilised in the project, we have taken advantage of the NC3Rs Experimental Design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda). This free online tool helps to achieve the best experimental design to answer one's question efficiently, minimising the number of subjects (mice) necessary. Moreover, we have considered the PREPARE guidelines (https://norecopa.no/prepare) to ensure we followed the best available recommendations when planning our experiments. The PREPARE guidelines are designed to help scientists consider all those factors that may help to achieve the most efficient experimental design. Finally, we considered what our goals were, and we planned how we would like to analyse data and report experiments, in order to guarantee an adequate experimental design. In order to plan the analysis and reporting phase of the project we followed the ARRIVE guidelines (https://norecopa.no/3r-guide/arrive-guidelines).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In order to further optimise the number of mice utilised, we will performed stringent in vitro screening of our genetically modified macrophages before testing them on mice. Therefore, not all candidate genetically modified macrophages will necessitate of in vivo testing, but only a small fraction of them. Moreover, we will share tissue between collaborating scientists. This will reduce the chance of needless duplication of experiments. Finally, live imaging procedure will allow us to follow macrophage cell therapy homing in various tissue over time. This approach will allow us to follow the same mice in time, without having to cull different mice at the different time points post-therapy injection. In some very specific cases, in experiments designed to understand how animals respond to a range of doses of a particular drug, it might be possible to re-use the same animal by re-testing a range of doses over a time period. This approach may reduce the number of different animals required for dose-finding procedures. In other dose-finding experiments with pharmacological agents/drugs, existing data from control treated mice in older studies (i.e. historical controls) may be used as the comparator group, but only when the drug effect is very reproducible, and does not change between different batches of animals. These additional measures can reduce the overall number of animals required in this project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Acute and chronic liver disease models chosen for this project are very well characterised in the scientific literature. One of the advantages of using these methods is that the potential adverse events are very well known, and therefore we can efficiently monitor our animals, spotting them early at their onset and act upon them early to minimise animal suffering. Further, the high standardisation of the models means that we have already implemented stringent monitoring methods based on weight loss and clinical signs (hunching, squinting, withdrawn behaviour, etc) for models of TAA-induced, CCl_4induced liver fibrosis and APAP overdose. Plus, the

REDACTED have implemented state-of-the-art animal housing facilities, including environmental enrichment, which will

minimise stress for the animals during protocols. When we will work with immunodeficient mouse strains, it is necessary to provide antibiotics to reduce infection risk during certain procedures, which will reduce any unnecessary suffering, distress, or lasting harm.
Macrophage cell therapies have been administered in the past, in proof-of-concept studies, either in the spleen or through the hepatic portal vein. Both these routes of administration are invasive and require surgery. Instead, we use intravenous (i.v.) injection to deliver macrophages to animals. This way, we use models that faithfully reflect the design of the ongoing clinical trials where non-engineered macrophages will be injected intravenously in patients. Intravenous delivery is a safe and well-tolerated delivery route and will minimise the stress for the mice during injection. Further, mice will be carefully monitored after macrophage injection to ensure no severe side effects develop.

Why can’t you use animals that are less sentient?

We cannot use mice during embryonic development as they would not be a faithful representation of acute or chronic liver injury, pathologies that typically develop during the adult life in humans. We cannot use more primitive animals such as zebrafish and drosophila because they only partially recapitulate the complex environment in which macrophages will be injected when used as cell therapy. Finally, we cannot use mice that are permanently anaesthetised because we have to follow animals up for several weeks after macrophage cell therapy injection to determine how effective the treatment is. Induction and regression of liver fibrosis usually takes several weeks for detectable changes to occur.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In order to achieve the best results and minimise the welfare costs (harms) we will:

1 - As standard, house any animals subjected to anaesthesia in a heated cabinet to provide thermal support to aid recovery

2 - Provide thermal support to animals during any procedure that reduces body temperature (e.g. following paracetamol-induced liver injury) or if an animal becomes sick

3 - implement frequent checks, include weighing animals twice-a-week or more, as recommended by the local Named Veterinary Surgeon (NVS). Any animals about to exceed moderate severity will be humanely culled

4 - ensure adequate training of technicians and other personnel performing experimental procedures, so that they could be aware of the potential side effects and how to minimise pain and stress during the procedure itself

5 - administer analgesic drugs if recommended by the NVS

6 - implement a clinical scoring system that considers all aspects of the mouse behaviour that could indicate suffering, as advised by our NVS

7 - ensure that, whenever possible, the same person handles the mice when a protocol requires repetitive handling and manipulation (for example injecting CCl_4 twice-a-week for six weeks). This will decrease the stress for the mice, and will standardise monitoring after injection

8 - use appropriate anaesthetics and utilise them properly during surgical procedures

Importantly, the animal unit routinely uses appropriate environmental enrichment and handling techniques. These actions guarantee that the level of stress are kept at a minimum during the duration of the protocols.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow constantly the NC3Rs and Norecopa websites in order to have the most up to date knowledge around experimental design, monitoring and minimisation of discomfort during experiments. We will also use databases such as PubMed or Google Scholar to search for the most up to date literature on our topic.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our group will continuously refer to the NC3Rs website to stay informed on best practice in animal research (https://www.nc3rs.org.uk/). Further, we will participate to the local NC3Rs symposium, both actively by presenting our research and as delegates. Finally, we will participate to the personal licence holders' local annual refresher sessions organised by REDACTED at the establishment. We will implement any new recommendations by interacting with our local veterinary services, and by reading the relevant literature on the topic, in order to gain a better understanding of the practicalities around the change or refinement of a specific practice.

Home Office

NON-TECHNICAL SUMMARY

106. Mapping mechanisms for energy homeostasis in rodents

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Diabetes, Islets, Glucose, Homeostasis, Energy

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this research programme is to look at why and how diseases related to loss of control of energy balance- principally diabetes and obesity- develop. In this project, mouse models will be used to look at the contribution of a protein that is a fuel sensor, and the products of genetic variations that were identified as conferring disease risk in humans, in disease progression. Such studies are important as it is known the reasons behind disease is different between individuals so understanding the individual risk (by looking at the contribution of products of genetic variations) and more general risk (contribution from aberrant fuel sensor function) will provide a better picture of these diseases and how treatment may be tailored to the individual.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Our data indicate a fuel sensor is involved in regulating blood glucose levels and food intake/appetite. It is a particularly difficult protein to study and we have the necessary knowledge, experience and developed the tools that will allow us to study this potential drug target. Understanding the contribution of genetic variations that lead to disease will allow us to better tailor treatment for individuals based on which genetic variations they carry in their genome. This work is important as c.100 variations have been associated with diabetes/obesity, with more on the way, and we are very far behind in establishing whether/how these variations contribute/lead to disease. The work proposed in this project will chip away at this colossal work load

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project? We expect

to use mice (10000) and rats (500) over the course of the project licence

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

In brief we will be inducing obesity and diabetes with administration of diets with high caloric value and/or chemical/genetic manipulation. We will then try to reverse obesity and/or diabetes using drug treatment, and/or alteration of diet regiment. Treatment and monitoring of diabetes will require the use of procedures similar to those used in human patients. Animals will be humanely killed at the end of the project period. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The maintenance of normal energy balance requires the interplay between hormonal secretion from, and hormonal action on, different tissues. Neuronal outputs from the brain in response to changes in hormonal signalling and nutrient availability also modify the net effect on energy balance. Such complex interrelations cannot be reproduced in vitro and require a whole living organism. Although we do most of our work on cell lines and freshly isolated cells, we ultimately need to validate the systemic effects in the whole animal. Validation of the efficacy of drug targets can only be assessed in the whole animal.

Reduction

Explain how you will assure the use of minimum numbers of animals.

For most of the quantitative experiments, sample sizes will be set using careful statistical analysis. We will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from other published reports). Usually 6-8 animals per treatment group are sufficient to obtain the required results

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice are the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. Rats give a better yield of blood and tissues per animal than mice and are preferred when we do not need to use transgenic animals. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals. Where appropriate, pain relief will be provided

Home Office

NON-TECHNICAL SUMMARY

107. Measuring brain activity of fish during humane slaughter

Project duration

4 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

No answer provided

Animal types	Life stages
● Tilapia (Oreochromis niloticus)	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The aim of the project is to develop and apply a method to measure brain activity of fish around the time of slaughter, to allow the development of stunners that render them unconscious immediately and until they are killed.

A retrospective assessment of these aims will be due by 24 February 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In 2010 it was estimated that between 37 and 120 billion farmed fish were slaughtered. Ensuring humane slaughter of farmed animals, including fish, is an essential part of ethical food production. Humane slaughter requires that the animal is rendered immediately unconscious and does not regain consciousness until it is dead, this mostly requires some form of stunning. At present there is only effective pre-slaughter stunning for a small proportion of farmed fish. To ensure that the stunners are effective we have to measure the brain activity of the fish. This project will develop and apply methods to measure the brain activity of fish around the time of slaughter.

What outputs do you think you will see at the end of this project?

The project should result in new products leading to commercial opportunities and publications affecting worldwide improvements in animal welfare. It will produce a non-invasive (externally applied) system to monitor brain activity in a variety of fish species. It will facilitate the development of humane stunning equipment and allow evaluation of existing stunning systems.

Who or what will benefit from these outputs, and how?

The ability to monitor brain activity in animals around the time of slaughter is essential to ensure that the stunning is effective, and that they do not regain consciousness. The findings of the project can be rapidly implemented due to a rising demand in the sector. This project will benefit the welfare of farmed fish and farmers using the equipment and all those interested in the ethical production of farmed fish.

How will you look to maximise the outputs of this work?

This research proposal is a collaboration between industry, academia and the wider supply chain. The project will expand and refine our ability to humanely stun fish by widening the range of environments and fish species for which effective practical and objectively validated humane stunning systems can be supplied. Following scientific validation of stun parameters, full scale prototype stunning equipment will be installed in fish processing plants for additional commercially important species.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will initially use trout or salmon, since these are the species on which the majority of work has been conducted and published. Subsequently we will focus on the commercial target species: initially we will work with tilapia and subsequently if time and resources permit pangasius catfish.

Typically, what will be done to an animal used in your project?

Most of the fish will be anaesthetised and have their brain activity recorded. Some will then be stunned (rendered immediately unconscious) using an electrical field in water.

What are the expected impacts and/or adverse effects for the animals during your project?

We anticipate that the vast majority of the fish will experience no more than routine handling before being anaesthetised or stunned. Anaesthesia is a common husbandry practice based on addition of anaesthetic to the water. While this does cause a mild stress response it does not appear to cause any pain, suffering, distress or lasting harm. The fish that are stunned will be rendered immediately unconscious (<1 second) and will not regain consciousness before they are killed. However, in order to identify the effective lower limit of stunning it is possible two fish may suffer a brief electric shock before being humanely euthanised.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For the test trout or salmon 100% will be sub-threshold or mild.

For each of the two target species (tilapia (n=150) and Pangasius (n=100)):
Non-recovery 33% (Tilapia), 50% (Pangasius)

Mild greater or equal to 63% (Tilapia), 44% (Pangasius)

Severe not greater than 4% (Tilapia), 6% (Pangasius)

All the fish will be humanely killed by a schedule 1 method at the end of the procedures.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 24 February 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project aims to develop methods to measure brain activity around the time of slaughter to make the slaughter of fish more humane. This can only be achieved through the use of live fish.

Which non-animal alternatives did you consider for use in this project?

Where possible we will use dead fish for testing our equipment but once we reach the stage of measuring brain activity, we need to revert to live fish.

Why were they not suitable?

Brain activity can only be measured in live animals.

A retrospective assessment of replacement will be due by 24 February 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

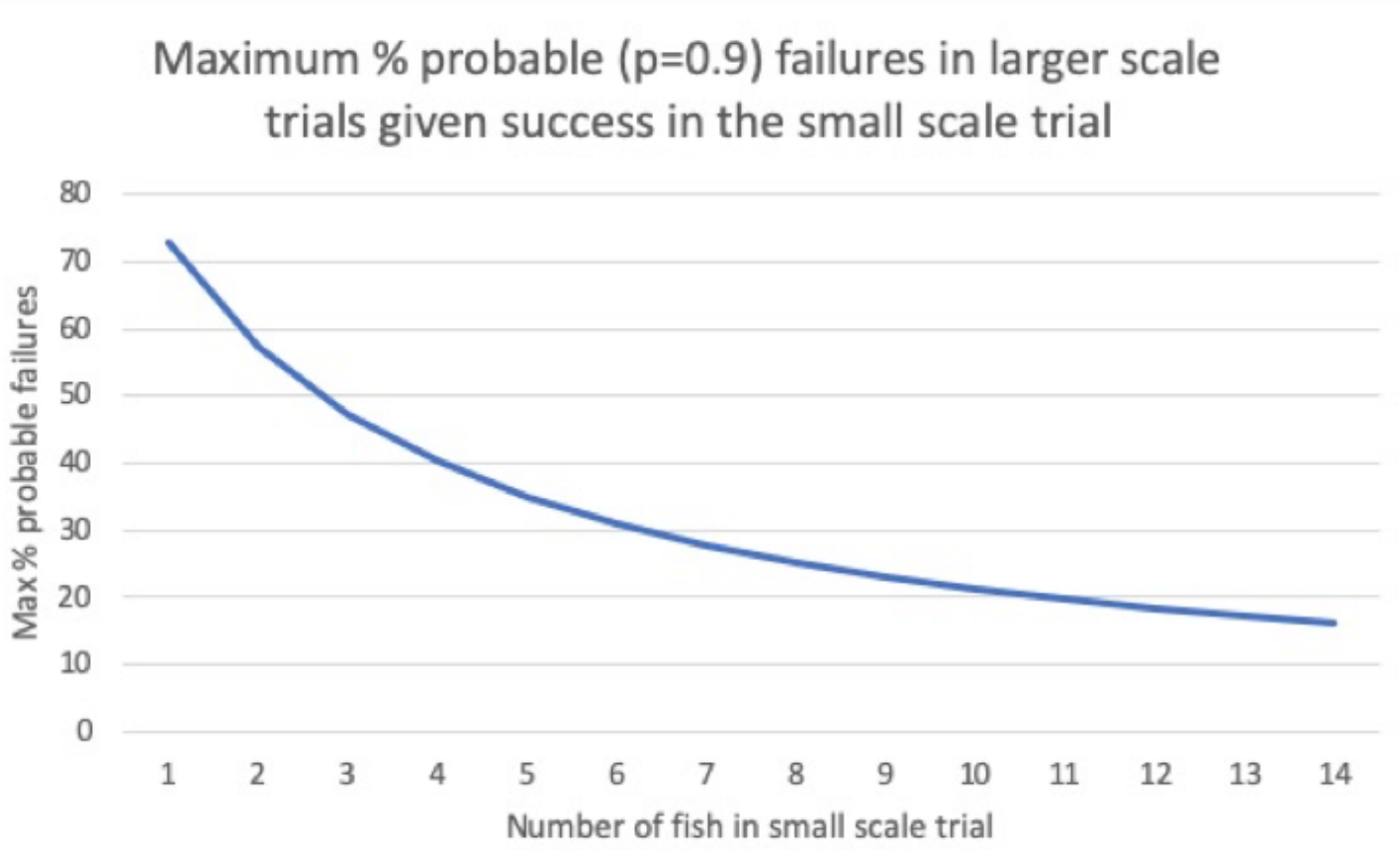
Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

the UK Animals Scientific Procedures Legislation (ASPA), Development of systems and procedures will be conducted with individual fish. Once we have systems or which appear robust at the individual fish level, we will use 6 fish per trial.

Success in 6 fish gives a reasonable compromise between power and numbers of animals used. Success in 6 fish suggests that failure rate in larger scale studies will be less than 31% (p=0.9), see graph.



Proportion in a single sample using Wilson's method (Altman, D (2000) Statistics with Confidence: Confidence Intervals and Statistical Guidelines, 2nd Ed. BMJ Books, 254pp.)
Eventually experimental trials will result in stunning parameters that will be used to inform commercial processes. At this stage although the activities will not be controlled under the UK Animals Scientific Procedures Legislation (ASPA), we will continue to monitor the efficacy of the stunning system to detect low prevalence problems that are unlikely to be detected in small scale experimental studies.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

There is an inevitable compromise between reducing the number of animals and improving confidence in the results. We worked with a professional statistician to reach such a compromise. When using stunning equipment prior to slaughter there are some problems that only occur very rarely. We will not try to detect such problems under experimental conditions, since this would require the use of a very large number of fish most of which would not produce any useful information. Rather we have another phase of the project which will monitor large numbers of fish in commercial systems (outwith the UK). This will not be experimental and therefore will not covered by ASPA but will allow us to detect issues that only occur very occasionally e.g. 1 in 1,000 fish or even less frequently.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The research group has a great deal of expertise in all the key areas, minimising the need for developing new methods or equipment. In addition, we have an agreement with international groups which will allow us to exchange information and work in each other's laboratory. As a result, we will avoid duplication of effort in the two sites and both groups will benefit from advances made by either group.

A retrospective assessment of reduction will be due by 24 February 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use the external application of electrodes to the head of fish to measure brain activity. This is far less harmful or distressing than the alternative surgical implantation of electrodes.

Why can't you use animals that are less sentient?

We are developing systems for monitoring the slaughter of commercially important species and therefore need to work with those species.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Humane slaughter is an essential part of ethical food production and Electroencephalography (EEG) or measurement of brain activity is essential for determining if methods of stunning are humane. Currently accurate EEG measurement in fish requires surgical implantation of electrodes. The insertion of electrodes directly into the brain of fish, may result in pain and discomfort, requires surgical anaesthesia, delicate surgery, and that the animals are restrained post-surgery. The method being developed and applied here will simply require the application of a suction cap to the head of the fish. While the fish's activity will then have to be restricted, they will not have to be tightly restrained. This not only drastically reduces the severity of the procedure but will also allow data to be collected under commercial conditions.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will apply the NC3Rs guidelines - Institutional framework for the 3Rs, and Responsibility in the use of animals in bioscience research.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The hosting institution runs a compulsory program of training and refresher courses to ensure all staff using regulated procedures are aware of current developments. The Animal Welfare and Ethical Review Board and Named Information Officer also circulate any relevant information on a regular basis. The licence holder will also maintain a watch on the relevant literature. **A retrospective assessment of refinement will be due by 24 February 2025**

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future

work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

108. Mechanisms and treatments of eye disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Eye disease, Blindness, Retina, Photoreceptors, Gene therapy

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, aged
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of the proposed project is to develop treatments for eye disease, especially those that affect the function and/or survival of the retinal pigment epithelium (RPE), neural retina (including the optic nerve) and retinal vasculature.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to find novel treatments, and improve the current ones, to beat sight loss. Partial sight and blindness in the adult population impose a large economic cost on the UK economy. Eye diseases become more common with the ageing population, vision impairment associated with conditions such as age-related macular degeneration (AMD) and retinitis pigmentosa (RT), where the retina, the light sensitive layer of tissue at the back of the eye, degenerates with age, compromises the quality of life and limits social interaction and independence. It has also been shown to interfere with the person's ability to care for themselves and others indicating a need for community and vision-related support. Aside from these socio-economic considerations, it has been reported that after cancer, people fear losing their sight more than any other medical condition. Besides, the degree of debilitation that sight loss can have on societies is important to consider, especially when effective treatments for many ocular conditions are limited. Vision loss can affect one's quality of life, independence and mobility and has been linked to falls and injuries, which in turn can have a big impact on mental health and wellbeing.

What outputs do you think you will see at the end of this project?

The main output of the project is the development of new and improved therapies for diseases and disorders of the eye. This will contribute to the current understanding and applications of treatments and therapies in the field. Our group has an established track record of successfully translating laboratory work into clinical trials. it is expected that any positive results of this project will be used to perform human clinical trials. In the future, this will lead to new or improved therapies for diseases and disorders of the eye and would therefore provide direct benefit to patients with eye disease.

Work under this project will also produce new information in the field which will be published, contributing to expanding knowledge in the field.

Also, the treatments assessed under the project will result in the development of gene and cell replacement therapies for the eye

Finally, products such as novel vectors for gene therapy will be generated for use in clinical trials. We are currently working on two novel viral vectors used for gene therapy in the eye, one for treatment of *CDHR1* gene mutations in the retina called (AAV8.CDHR1), and another on RNA editing of the *USH2A* gene. We are also planning to generate a novel Base Editing construct for the treatment of ocular conditions caused by single nucleotide mutations.

Who or what will benefit from these outputs, and how?

This project will develop new and improved therapies for diseases and disorders of the eye, providing a real benefit to human health and wellbeing, and also to fellow scientists and researchers in the field. Work in animal models has already been very successful in understanding the mechanisms of eye disease and in the development and testing of new treatments.

Benefits from the work under the project may be seen in the short-term as well as the long-term. Knowledge and information in the field produced by publications will be realised in the short-term and will benefit the scientific

community. Treatments and therapies discovered under the project will be taken to human trials and it might take several years to see the final result, however, if successful, this will provide a real benefit for people who suffer from debilitating eye conditions and it could have a big impact on their lives.

How will you look to maximise the outputs of this work?

We aim to explore novel therapeutic approaches of gene editing in the eye using molecular biology tools, such as clustered regulatory interspaced short palindromic repeats, known as CRISPR, and validate its efficiency and specificity as a gene editing tool as well as its safety as a therapeutic agent. We aim to achieve this by establishing collaborations with experts in the field of in vivo application of CRISPR tools. These collaborations will help to validate effective treatments of ocular diseases that are caused by mutations in specific genes. Current knowledge of efficacy and safety of such therapeutic approaches is limited, especially in the eye, and validation of any approaches, including unsuccessful ones, will vastly contribute to disseminate knowledge, expand our understanding of treatments and help to achieve our main goal of developing new and improved therapies for diseases and disorders of the eye.

Species and numbers of animals expected to be used

- Rats: 500
- Mice: 36 000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice, and rats, are the species of lowest cognitive ability that are suitable for eye research and are highly appropriate because they are well defined model organisms and well characterised disease model strains with inherited ophthalmological dystrophies already exist. We use animals at different stages of development to model different ocular disorders. For example, for ocular conditions that have a developmental origin, such as nystagmus, a significant eye disease with uncontrolled eye movements, it might be necessary to use neonates to study potential causes of the disease. For other diseases, especially those with late onset retinal degeneration, adult animals will be a scientifically more appropriate model to study the disease.

Typically, what will be done to an animal used in your project?

Typically, we aim to assess efficacy and safety of different therapeutic approaches to treat eye disease, such as gene therapy, by injecting animals with novel recombinant vectors that carry a correct copy of a mutated gene or a therapeutic reagent with a specific pharmaceutical property, injections normally performed in the eye. Changes in ocular behaviour/phenotype are normally assessed by a combination of techniques, such as eye imaging and visual electrophysiology, two weeks after injections or treatment. The imaging/electrophysiology can continue for a period of time (up to 12 months sometimes) to assess changes in the ocular behaviour and/or eye or retinal morphology, which can indicate efficacy and safety of the therapy applied.

What are the expected impacts and/or adverse effects for the animals during your project?

Adverse effects of the techniques used in the project can differ according to the technique used. The main impact on animals in this project is visual impairment/blindness, which in itself is not known to affect the animal's welfare and ability to thrive. the visual impairment/blindness once it starts, it will last a lifetime. The techniques performed in the project involve injecting therapeutic reagents into the eye so there is a risk of inflammation and infection in the site of injection. Such adverse effects, if occurred, are not expected to last a long time (a few hours).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In general, the expected severity of the protocols performed in this project is mild, this is for the majority of animals (approximately 95%). A small proportion of animals (less than 5%) might experience a moderate severity due to performing more than one procedure during their lifetime.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals must be used for this project since the development of therapies requires an assessment of efficacy and safety in live animals. The eyes of lower, non-mammal species are quite different from those of humans and so do not make good models to use to test potential clinical therapies in.
Wherever possible, treatments will be initially investigated in non-animal alternatives such as cells and tissue from human donors.
Which non-animal alternatives did you consider for use in this project?

We considered using human retinal tissue for retinectomy (surgical removal of small retinal tissue during routine surgery) as an *ex vivo* alternative approach. We also considered using isolated cells from patients skin biopsies as an *in vitro* alternative approach. We also routinely culture immortalised rodent and human cells lines of retinal or otherwise neuronal origin.
Why were they not suitable?

Animals must be used since the development of therapies requires *in vivo* assessment of efficacy and safety. For instance, in the case of intraocular/retinal inflammation (which occurs as part of retinal degeneration, in response to retinal gene therapy, or as part of uveitis), the systemic (humoral) immune system plays a central role in driving the inflammatory response. Therefore, a whole organisms-based model is required to study the mechanisms of retinal inflammation and test potential therapies.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers have been estimated according to previous experience from previous licences.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Animal numbers will be kept to a minimum by using the most appropriate experimental design and statistical analysis. Wherever possible we will use technologies, such as imaging, to enable longitudinal studies in the same animal. Avoiding systematic error/bias and using adequately powered, multi-factorial experiments will help to reduce the final number of animals that are used. We use our experience of procedures and protocols to enable reliable estimation of the likely experimental and biological variation to be used in power calculation for determination of appropriate sample size (using http://statpages.org/). We also perform experiments that are 'proof of principle' and/or qualitative descriptions (e.g. test if a vector is capable of transducing particular retinal cell types). In this case, statistical analyses need not be applied and a very small number of animals (2-3) will be used.
Pilot/feasibility studies are also used, especially where from novel treatments or techniques.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have reduced animal use by establishing an *ex vivo* culture of mouse retinal tissue. This will never be able to fully replace in vivo studies and does not completely remove the requirement for animal use, as the mouse line/s used to generate tissue must be maintained. However, procedures on live animals are reduced (animals humanely killed before extraction of tissue) and several retinal tissue preparations can be made from one eye, allowing for experimental replication and/or multiple treatments to be assessed.

Using ocular imaging in mouse lines in which subsets of retinal cells are genetically fluorescently labelled means we can to non-invasively quantify retinal cells in vivo. We can longitudinally assess cell numbers in the same individual, quantify ongoing retinal degeneration and evaluate the effectiveness of therapies to prevent cell loss. Repeated measurement in the same individual dramatically reduces the number of animals used compared to histological techniques and does not suffer from inter-animal variability. More generally, we can often apply treatment to one eye and use the other as an untreated/sham-treated control. This reduces animal numbers, by abolishing the need for a separate cohort of control animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice, and some extent rats, will be used during this project. Rodent models, and particularly mice, have become the most widely used models of human disease. These small mammals are easy to manage in a laboratory environment, and multiple mouse mutants of retinal disease are already recognized or can be generated relatively easily for investigations. Also, in vivo transfection or silencing of specific genes in mouse retina or in vitro transfection of retinal explants, using electroporation (Matsuda and Cepko, 2004) allows rapid examination of genes and variants.
Why can't you use animals that are less sentient?

For treatments to have biomedical relevance and applicable to humans, a mammalian model organism must be used. Mice are the species of lowest cognitive ability that are suitable for eye research and are highly appropriate because they are well defined model organisms and well characterised disease model strains with inherited ophthalmological dystrophies already exist.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For models of retinal degeneration, such as the Rhodopsin knockout strain, the loss of rod photoreceptors is the main cause of visual impairment/blindness, which is caused by a faulty rhodopsin gene. One effective route to slow down the loss of the rod photoreceptors, or stop it completely, is treatment with cell replacement therapies. Recent advances in gene therapy in the eye, particularly the applications of CRISPR gene editing, meant that targeting disease causing mutations is achievable by injection of therapeutic agents into the retina. Subretinal injections is a well-established technique that provides effective delivery at the target site of the disease. To minimise potential harms to the animals as far as possible, we use the best possible equipment for experimental eye surgery and assessments. We often use clinical grade equipment that has been modified for animal use. We make sure our methods are conducted within the internationally recognised guidelines for animal eye research, laid down by the Association for Research in Vision and Ophthalmology (ARVO). We also make sure anyone conducting complicated procedures is highly skilled and adequately trained. Many people working on the licence will be clinical ophthalmologists, specialised in human eye surgery and transferring those skills for use in animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

- The Laboratory Animal Science Association (LASA).
- The guidance on aseptic surgery (LASA).
- Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 NC3Rs
- ARRIVE guidelines.
- NC3Rs responsibility in the use of animals in bioscience research guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We aim to periodically follow guidance on advances in the 3Rs from the 3Rs website. Also, at a local level, we regularly attend a departmentally arranged 3Rs meeting where different aspects on the applications of 3 Rs are discussed with local experts of animal research in the university.

Home Office

NON-TECHNICAL SUMMARY

109. Mechanisms in very early mammalian embryos

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- (e) Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work **Key words**

fertilisation, gamete-to-embryo transition, preimplantation development, biotechnology, biomedical model

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand the process by which the gametes transform into an embryo during fertilisation using the mouse as a model.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Today, there is no comprehensive model of the gamete-to-embryo transition in any species, so this work addresses a fundamental biological question: how does embryonic life begin? The model we use is the mouse owing to its biomedical relevance and because many of the key processes occur too rapidly in other models (*e.g. Xenopus*, flies or zebrafish) to be captured.

What outputs do you think you will see at the end of this project?

In general terms, the work addresses the mechanisms underlying the extraordinary change that occurs when the gametes (sperm and egg) transform from cell death into a one-cell embryo during fertilisation. This work is fundamental in nature but promises to impact human and veterinary clinical medicine because it links so many important biomedical processes. These include cancer, the regulation of body mass, the new idea that acquired traits can be inherited (highly clinically and economically relevant to the inheritance of acquired obesity), and regenerative medicine. For example, tomorrow's regenerative medicine requires that we have a good understanding of how cells control their fate, but no such understanding exists today. The potential of regenerative medicine to revolutionise certain areas of health-care is recognised internationally. The approaches we adopt contribute to this and promise to reduce animal use by streamlining genome manipulation and illuminating mechanisms of reprogramming. Advances in genome manipulation should be applicable to larger animals, such as pigs that are favoured by active collaboration in this area by sharing knowledge obtained in the mouse. Early embryonic pathways and components may suggest new explanations of idiopathic human infertility. As the outputs relate to specified Objectives, they are as follows:

Objective 1. To determine chromatin changes in mouse one-cell embryos. Outputs:

1. Identification of chromatin remodelling activities required in one-cell embryos for full-term development.

2. Determination of altered chromatin remodelling in atypical embryos to help delineate normative remodelling following fertilisation.

Objective 2. To determine transcriptome changes in mouse one-cell embryos. Outputs:

1. Determination of the precise kinetics of embryo genome activation (EGA).

2. Identification of genes participating in EGA.

3. Identification and characterisation of factors that activate EGA genes and their interactions.

4. Characterisation of epigenetic inheritance as manifest in preimplantation embryos.

Objective 3. To study how changes in mouse one-cell embryos are integrated. Outputs:

1. Demonstration of how cell cycle progression (meiotic exit) impacts chromatin remodelling.

2. Evaluation cross-talk between embryo processes; for example, how the protein Emi2, maintains ensures that cell division occurs normally.

3. Evaluation of components that regulate the normal cell division.

Objective 4. To harness processes in mouse one-cell embryos for new methods of genome and proteome manipulation. Outputs:

1. Refined genome editing using next-generation editors and base and prime editors.

2. Refinement of homology-directed repair-mediated editing.

3. Establishment of a protocol for genetic code expansion for inducible protein expression *in vivo*.

Who or what will benefit from these outputs, and how?

The work covered during the lifetime of the license will help to fill a fundamental knowledge gap concerning how one-cell embryos are formed. This is expected to have broad implications that will impact academic and commercial research communities including post- and under-graduate students (*e.g. via* the revision of text books) whose interests include embryology, cellular potency regulation, development, bioinformatics, epigenetics and stem cells. In the near term, the project will impact research communities studying fundamental aspects of gene regulation in human one-cell embryos, but it will subsequently contribute to greater awareness of human development locally, nationally and internationally. Over the longer period, extending from the duration of the work to years beyond it, knowledge generated here will also inform new healthcare messages, interventions and treatment regimes, with beneficiaries that include health care professionals, private and public health institutions (NHS, social services), policy makers (Department of Health, Health Authorities), charities (*e.g.* WHO, Diabetes UK, BHF), pharmaceutical and biotechnology companies. Overall, this potentially includes all groups whose work touches upon healthy development, since all development begins with a one-cell embryo. It will directly impact assisted reproductive technology (ART) by suggesting new diagnostic markers and treatments. Mechanisms underlying non-classical inheritance may operate in multiple (or potentially all) circumstances at the one-cell embryo stage, so this work will accordingly be relevant to those working on the inheritance of acquired traits. The work will illuminate mechanisms of embryo formation, thereby informing practical improvements to cellular (regenerative) medicine strategies that involve changes in cell fate potential. Information gained by the proposed work may provide vital information for human genome editing.

How will you look to maximise the outputs of this work?

Outputs will be maximised by publication in high-profile journals. Seminars, policy-makers and the commercial sector will be used to communicate to academic and clinical stake-holders. International meetings will grant the work a broader scientific audience that includes clinicians, those engaged in translational research. Wherever possible, the media will be asked to promote awareness of human early embryonic development and its healthcare policy implications. New technical advances applied to human early embryos are likely to affect everyone in the coming decades, so the work pays attention to communicating often impenetrable jargon by engaging broad publics, for example at TEDx and the Progress Educational Trust internationally. Outreach will be extended by contact with post-graduate and final year undergraduate students in seminar series, lecture courses to final year undergraduate students and as a recent STEM Ambassador with frequent presentations to A-level students. Research news will be disseminated to local, national and global audiences via university communications and events offices as well as communication through the internet.

Species and numbers of animals expected to be used

- Mice: 20,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Since very early embryonic development (the subject of this work) is distinctive in mammals such as humans, the choice of model species is restricted, and commonly-used model systems such as frogs, zebrafish or invertebrates such as flies are not applicable. In these non-mammalian models, the start of embryonic genome activity (known as EGA in mammals and the mid-blastula transition, MBT in nonmammals) occurs in embryos comprising many hundreds of cells, whereas in human and mouse it occurs much earlier. There are other differences. For example, in frogs, nuclear size scaling contributes to the timing of EGA, but no such scaling is reported in mammals. Multiple differences also exist between mouse and frog one-cell embryos. The mouse (*Mus musculus*) is a model because it is a genetically and embryologically tractable system enabling micromanipulation, genetics, embryology, transgenesis, gene targeting and imaging. These are some of the reasons why the mouse is a widely accepted model of health and disease in livestock and other larger mammals that may be biomedically important (*e.g.* pigs), as well as humans. Indeed, the mouse is a model of human pathobiology that will allow disease traits produced by embryo interference to be mapped onto human conditions. The mouse system is likely to be particularly amenable to revealing links between the early embryo, body mass control and cancer, opening new avenues to their study. The outcome is expected to enhance not only disease modelling but provide a clear line of sight to clinical diagnosis and therapy.

Typically, what will be done to an animal used in your project?

Most will be used for the production of sperm and eggs (gametes); this project is mostly concerned with manipulating and monitoring gametes and preimplantation embryos. For a comprehensive analysis of these early processes, it is necessary to show how they are manifest later in development, including in adults.

What are the expected impacts and/or adverse effects for the animals during your project?

The vast majority of mice used in the work of this license are expected to be healthy. Phenotypes of a subset are not predictable, but we anticipate that some may develop tumours or experience metabolic abnormalities (*e.g.* weight gain). Animals will be carefully monitored by ourselves and staff, and any signs of poor health will trigger the involvement of the NVS.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The vast majority (>99%) of animals are expected to be healthy and not to experience any enduring discomfort. The small proportion exhibiting ill-health are expected to belong to the GAA (moderate) category, expressing transgenes corresponding to transcripts present in early embryos.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Wherever sound alternatives to animals are available they are taken, including predictive computer algorithms and cultured cell lines. However, there is no accepted in vitro source of mouse gametes that are central to the proposed work. Recent studies have reported 'in vitro' gametogenesis, and whilst these studies are extremely impressive, spermatozoa have not been produced (post-meiotic precursors, spermatids, were obtained). The production of eggs required *ex vivo* material and the resulting oocytes produced abnormal offspring. Moreover, the efficiency of full-term development supported by either set of gametes (combined with naturally-produced counterparts) is very low - a maximum of ~4%. These features of *in vitro* gametogenesis make it unacceptable for the work of the current proposal; it introduces unknown and non-trivial variables. It is therefore unavoidable that gametes for the proposed work are recovered *ex vivo*.

Which non-animal alternatives did you consider for use in this project?

We have considered gametes produced from cells cultured *in vitro* as described above. The proposed work is complemented by other, non-animal approaches, but they are not alternatives: if there were alternatives, they would be used.

Why were they not suitable?

There are no alternatives. The recapitulation of sperm and egg generation in mammals *in vitro* has never been completely achieved and what has been done is extremely inefficient. The use of normal, healthy gametes completely capable of supporting full-term development is a *sine qua non* in the present work.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimation is based on extensive experience on early embryogenesis, accommodating consultation with statisticians and input from experts in this area. It assumes that there are two postdocs whose main occupation is performing micromanipulation. For experiments in particular requiring oocytes and embryos produced following *in vitro* manipulation, it assumes an average of ~20 oocytes are obtained per female and that over the course of five years, there are two phrase or equivalent, requiring 25 mice per week (48 weeks per working year) to produce oocytes mainly for *in vitro* maturation (IVM) and/or micromanipulation such as intrcytoplasmic sperm injection (ICSI) and/or *in vitro* fertilisation (IVF). Oocyte and embryo manipulation and analysis are

central to the work of the laboratory. In many cases the oocytes and embryos will be genetically modified (GAA), for full cell biological and developmental evaluation of genes expressed in early (especially one-cell) embryos. The number of GAA required reflects a combination of factors, including their utility (*e.g.* Venus-Tubulin, or histone H3-mCherry mice can be used in multiple lines of work), fertility (some GAA do not breed well, requiring greater numbers), the efficacy of the mutation where it is generated *de novo* (*e.g.* by genome editing) as, for example, edits often do not inactivate their target gene, and developmental phenotype (some homozygotes result in embryo death, meaning that the mutation has to be maintained in heterozygotes, increasing the animal number required). This last point is germane because we are studying the roles of genes in early development. There are fluctuations in GAA mouse colony numbers due, in part, to the specific nature of the work focus at a given time. By way of example, we might expect one post-doctoral researcher (pdra) to average ten colonies (each of ten GAA) including genome-edited mice and transgenic lines including reporter lines (*e.g.* ubiquitously expressing fluorescent proteins); at 100 GAA/week, this totals 15,600 mouse weeks for one pdra over a typical three-year project grant.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The transgenesis and editing protocols that will be used minimise animal numbers by producing improved models containing fluorescently-labelled gamete proteins and utilising a highly efficient method, or by genome editing via a highly efficient method. The work promises novel and improved methods of genome manipulation in mice and larger mammals, and it can realistically be hoped that they will reduce requisite numbers of both. The genetically altered animals here will be mice. Where suitable lines already exist, animals will be obtained from the relevant supplier or re-derived by embryo transfer (Protocol 2) from our previous work. Otherwise, we will generate the required lines ourselves or, in the case of gene-targeted lines, in collaboration.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will institute an efficient breeding programme for Protocol 05, the best estimate of precisely how many animals is comprised of two components: numbers required for the production and consolidation of GA mice, and the number of GA mice required for the expansion of desirable lines. The Production estimate assumes 10 lines per year, each of which derives from 5 founders (F0). Crossing with C57BL/6 will generate 20 transgenic F1 and 40 transgenic F2 offspring, the minimum for analysis to yield transgene-expressing lines for brother-sister mating, to generate 12 F3 offspring. This yields 77 GAA/line/year. Expansion is necessary for a thorough analysis of transgene behaviour or where transgenes encode markers. The estimate again assumes that an average of 10 lines are maintained at any one time. We share tissue where possible and perform strain storage and archiving.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The vast majority (>99%) of cases will cause no pain, suffering or lasting harm to the animals. This is because

the methods are either non-invasive (*e.g.* breeding GAA) or standard protocols that are brief (*e.g.* superovulation) or refined and which would not succeed if distress and suffering had not been minimised (*e.g.* embryo transfer; efficiencies of >80% per transferred embryo are obtained). In the unlikely event that lasting distress becomes possible (e.g. because interference with a given gene unexpectedly produces a disease phenotype), the experiments are all set up to detect it at the earliest juncture, so that, with guidance from one or a combination of the HOI, NVS and NACWO, the appropriate response can be made and if necessary, the animal killed by a schedule 1 method.

Why can't you use animals that are less sentient?

In general, the phenotypic effects of altering components involved in early embryogenesis are not expected to exert adverse effects on adults - they are active far earlier in development - and cannot be studied except during healthy development to adulthood. Most of the animal experiments will be performed in preimplantation embryos .

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Methods will be adopted that have improved efficiency in mouse transgenesis and refined mouse genome editing. In addition, challenging research areas that are in error will terminate wasteful lines of animal research and help to refine the mouse model of early development.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will closely follow the new ARRIVE reporting guidelines (ARRIVE 2.0) published recently in PLoS Biology, which build on previous guidelines already accommodated in our approach and with respect to the NC3Rs (<https://arriveguidelines.org/news/new-arrive-guidelines>).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We are in contact with our regional NC3Rs Office and monitor the NC3R website (<https://www.nc3rs.org.uk>).

Home Office

NON-TECHNICAL SUMMARY

110. Mechanisms of diabetes-associated heart disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants.

Key words

Diabetes, Heart diseases, Heart attack, gene function

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to determine the fundamental role of genes involved in diabetes-associated heart

disease, in particular the effects of diabetes on the development of heart dysfunction. The objective is to develop the treatment strategies of heart dysfunction, particular in diabetes.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Diabetes affects 10% population worldwide. Approximately 57% diabetic people will develop some degree of damage to heart function. Diabetes not only affects structures and function of heart muscles, but also causes poorer prognosis after heart attack. This project will benefit diabetic patients suffering from heart disease by identifying new treatments to improve heart function.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The designed work will be conducted in mice for the duration of 5 years, which includes five thousand animals. Genetically modified mice will be used to study the role of genes in the heart.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Mouse models will be used to study the effects of both types of diabetes on heart disease. With both models, animals will have raised blood sugar levels, eat and drink more, and urinate more frequently than control mice. Animals are regularly checked, and cages are changed daily. We typically use streptozotocin to develop Type 1 diabetes on mice, in which loss of body weight may occur. Type 2 diabetes will be induced by high calorie food, obesity or skin irritation may occur. At any stage, if the mice display any distress or lose a lot weight, they will be killed humanely.
When checking blood sugar in models of diabetes, we may remove food from the cage for 6-18 hours, which may irritate the animals though water will be always provided. A small amount of blood will be collected under local anaesthesia to reduce stress in the animals.
Administration of pharmacological agents by injection or oral route (gavage) may cause transient stress. Where appropriate pain relief medication or local anaesthesia will be provided. The animals will be carefully monitored to ensure effectiveness.

Post-heart attack injury will be induced under terminal anaesthesia by ligation and subsequent untying one of the major blood vessels of the heart (coronary artery). These mice will not recover after surgery.
To assess cardiovascular function, mice will undergo a series of tests, including ECG for heart rate and rhythm, blood pressure which are not performed under sedation and cause little stress to the animals. Cardiac ultrasound will also be performed under general anaesthesia. These tests may be repeatedly performed, in which the animals will recover from general anaesthesia and be reused. The welfare of the animals in these longitudinal studies will be carefully monitored.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The anatomy and physiopathology of the heart in mouse is similar to the human heart. Diabetes is a whole-body disease, affecting hormone, immune and cardiovascular systems, which cannot be effectively tested in non-animal systems. Hence, there are currently no alternatives to the use of mice.
Cultured heart cells or isolated heart complement our understanding of gene function and treatment dose or efficacy. If any relevant non-animal alternatives become available during the course of the work, we will incorporate these in our projects.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will use statistical Power Calculations based on our previous experiments and experience, which allows us to minimise the number of animals.
We will use efficient and optimised breeding protocols to minimise animal number. In addition, the use of control animals can be reduced, because the variation between these mice is lower. Where possible control data from previous experiments may be used to minimise controls.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Gene changed mice are well-established models for studying the cause of heart diseases in human. Although gene changes may result in unexpected suffering, these occurrences are rare, and the mice will be monitored at all stages.
To minimise the harm to the mice, we will continue to use/improve these refined approaches and techniques, causing the least pain and distress whilst being able to meet our objectives.
Blood collection and administration of pharmacological agents by injection or oral route (gavage) will be performed with pain relief medication or local anaesthesia. Mice will be carefully monitored. We are familiar with the methods, and no adverse impact on animal welfare is expected.
Induction of post-infarction injury by ligation and subsequent untying of coronary artery will be carried out under general anaesthetic to ensure that the mice do not feel pain. The mice will not recover.
Echocardiogram will be performed under general anaesthesia, while ECG and blood pressure check are performed on conscious mice, which is free of stress. In the majority of cases, heart function tests will be performed under terminal anaesthetic from which mice do not recover.
At any stage, any mice showing signs of distress will be evaluated in consultation with the veterinary surgeon and killed humanely if the distress cannot be averted.

Home Office

NON-TECHNICAL SUMMARY

111. Mechanisms of Immunoglobulin G-related autoimmunity in chronic primary pain conditions, and pharmacological solutions.

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Chronic primary pain, auto-antibodies, epitopes, mechanisms, therapies

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

To understand the cellular and molecular mechanisms underpinning the pain phenotype in passive transfer models of chronic primary pain, and to establish potential pharmacological solutions.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Chronic primary pain conditions, including FMS (Fibromyalgia) and CRPS (Complex Regional Pain Syndrome) are amongst the most severe and disabling unexplained chronic pain conditions in humans, causing severe suffering and dramatic economic losses. In either condition only few patients attain long-term benefit from available drug treatments. New solutions are desperately needed.
Pain-sensitising serum autoantibodies in these conditions, can explain for the first time why patients get such pains. There is the potential for drug-, and diagnostic test development. However, to develop new solutions we now need to understand the cellular and molecular mechanisms by which the antibodies have their effect

What outputs do you think you will see at the end of this project?

Outputs will include a better understanding of these conditions, for example outlined in publications, novel leads to the development of diagnostic serum tests, and novel leads to drug developments
Where distinct epitopes are identified we hope that patents will be another output.
The respective UK and international Guidelines on information for patients with both conditions will be updated as appropriate (Royal College of Physicians and others) **Who or what will benefit from these outputs, and how?**

Patients and relatives, and employers will benefit as there will be better understanding of these conditions, which will facilitate diagnosis and acceptance - acceptance is still often an issue because many doctors, relatives or employers don't understand why patients would have pain without having a destructive problem – this is causing substantial suffering.
Patients and their relatives will further benefit as this research should lead to the development of diagnostic serum tests, likely within the 5 years of this license period, or at least to a clear pathway towards the development of such tests. Currently diagnosis relies on clinical criteria and is often severely delayed up to several years, leading to inappropriate treatment and suffering from not understanding what is wrong.
Patients, their relatives and the wider society may also benefit from the development of effective treatments arising from leads identified in this research. Current drug treatments are poorly effective, resulting in reduced activity, lost work days, and missed opportunities for personal development for many patients.

How will you look to maximise the outputs of this work?

We will work with various charities, patient organisations and Royal Colleges to enhance dissemination. We will also use the University Press Office and as appropriate approach national and international press to disseminate our findings.
We would publish unsuccessful approaches to avoid futile duplication - we have a good track record of doing this.

It is possible that by the time of the end of this license period commercial entities are being involved who can help promote new tests for example.

Species and numbers of animals expected to be used

- Mice: 3000
- Rats: 800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The use of rodent models for the assessment of chronic pain is the most appropriate scientific approach as rodent responses to a large number of pain stimuli have been well assessed in the literature and assessment methods have been shown reliable and valid; such data does not exist for any other species.
Excepting specific questions around paediatric pain, the use of adult rodents is the accepted method; in younger rodents the pain-sensing system is immature and any results cannot be translated into a better understanding of adult human pain mechanisms.

Typically, what will be done to an animal used in your project?

Animals will be habituated and will have at least one set of baseline tests of their sensory-motor function, in some cases they will be trained to perform some sensory-motor tasks to allow accurate recording of their sensory-motor function, no longer than 1 month before the operation (in the case of the CRPS protocol only) and/or start of human IgG injection (next step). They will all-together experience no more than very mild discomfort from such testing (optional step -most animals will undergo this step). Overall habituation and baseline testing are expected to *reduce* any distress the animals will experience in the establishment, and with later sensory-motor testing.
Animals in the CRPS protocol will then have a small, shallow hind-paw incision (obligatory step) under general anaesthesia (skin and muscle fascia), which will be sutured or glued. Most mice will exhibit some transient discomfort post-operatively (moderate). The wound will heal rapidly. In rare cases of wound dehiscence this will be repaired under GA.
In both protocols, either starting before the operation or after, animals will receive intra-peritoneal injection with human IgG or vehicle, maximally once a day. They may further receive application of a compound, either per subcutaneous/intraperitoneal/intravenous injection, or orally (no more than twice a day).
Both types of applications (IgG and compound) may be done on the same days and may continue daily for no longer than 3 weeks after the operation. This will cause no more than mild discomfort.
The animals may provide tail-vein blood, maximal frequency every two days not exceeding recommended withdrawal amounts, causing no more than mild discomfort.
The animals may then again be subjected to sensory-motor testing repeatedly - after the operation and/or first IgG injection - (no more than three tests per day), for maximally 21 days. These postoperative tests will cause no more than very mild obvious discomfort, although since this protocol is instituted to mimic a chronic pain condition (if human IgG is also injected), then if any mild behavioural abnormalities are seen their discomfort may be considered moderate with only mild signs.
At the latest 21 days after the operation and/or first IgG injection the animals will be killed, either with a schedule 1 method, or under terminal anaesthesia where blood may be drawn/paraformaldehyde intracardially injected.

What are the expected impacts and/or adverse effects for the animals during your project?

Operation

1. Animals which have undergone plantar incision may develop varying degrees of lameness. The hind paw on the operated side may be withdrawn into a protected position. The animals may walk with a limp. Recent studies have shown that the incision is fully healed in mice within 2-3 days, such that the wound is difficult to discern. The animals should be weight-bearing within 4 days; if they are not they will be killed by a schedule 1 method.

2. There is a small risk of dehiscence of the wound as animals will remove sutures (usually within one day) and as such wound glue may be employed. Wound dehiscence will be repaired once only under anaesthesia; animals with serious or repeated wound dehiscence will be killed. There is a minimal (less than 1%) risk of inflammation or infection after surgery.

3. Adverse effects from General Anaesthesia include hypothermia, dehydration, overdosing of the anaesthetic agent, however we will adhere to BSU standards for anaesthetic drug delivery and perioperative care, and the procedure is short, therefore we do not expect more than moderate effects. If signs indicating severe adverse effects unexpectedly emerge which cannot be rectified, the animal may be humanely killed - this will be decided in consultation with the NVS as appropriate.

Injections

Rarely injection site reactions or injection site inflammation may occur.
Mice may become irritable with repeated injections over time
In our experience repeated treatment with human IgG for less than 14 days does not cause more than mild adverse effects. Repeated treatment with human IgG for longer than 14 days can occasionally result in serum sickness (a reaction of the mouse immune system against the human protein), characterised by signs of general un-wellness.
Rarely systemic adverse events may arise from other compound treatments with, particularly novel substances. The effects of such novel test compounds are difficult to predict, but any compound for which no safety data in mice or rats exists may first be tested in non-protected species. We will consult the NC3r toxicology resource hub as appropriate.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The severity grade for all animals in the CRPS protocol is *moderate* because all animals (100%) will undergo a surgical operation, (1560 mice, 400 rats)
In the FMS model, the severity for those animals receiving IgG from patients is per definition moderate because they develop sensitivity that resembles a chronic pain condition although their behaviour on observation to the naked eye is normal or only mildly affected (e.g. moving/ rearing may be mildly reduced).
The expected severity for animals in the FMS model receiving healthy control IgG injection for mechanism research (n=440 mice, n=200 rats), or healthy control IgG plus saline vehicle injection for compound research (n=150 mice) are considered mild, i.e. a proportion of 41% of mice (i.e. of a total of 1440 mice), 50% of rats (i.e. of a total of n=400)

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We presently don't know how autoantibodies cause disease in FMS or CRPS but we have found that these human pain disorders can be reproduced in mice by 'passive transfer' of the patients' autoantibodies, i.e. the antibodies are taken from the patients' blood, purified, and then injected to the mice. This finding indicates that autoantibodies are responsible for the human disorders, and that they interact with the mouse organism similar as in humans, causing these pain disorders.
We need to use animals to achieve the first aim of our project, 'to better understand the mechanisms of autoantibody action', to understand at least the starting point of the autoantibody-related biological processes leading to disease.
The alternative would be to try and guess the relevant processes and hope that research using cell lines based on such guesses can identify the relevant mechanisms; such an approach has - to our knowledge - never been successful in any autoimmune disorder research where the antibody-target was unknown - posing risk that no new solutions for patients will be found. For example, we have identified specific autoantibodies against autonomic receptors in a substantial subgroup of patients with CRPS, using primary rodent cells. However while interesting, it remains unclear whether/how these antibodies specifically contribute to the disease process, or (more likely) whether they represent epiphenomena. This is hindering progress towards the development of novel therapies - this type of in vitro work has therefore reached its limitations.
We will pursue our second aim, to identify drugs that can ameliorate or abrogate the disease processes, only after we have been able to identify important disease mechanisms (as above), and only after - following appropriate advice, alternative methods (use of non-protected species or cells) to identify potential safety problems have been applied. At that point, the alternative to using animals would be to use these drugs in humans *without* efficacy or safety data in animals, which is not feasible. We will only use animals for as long as is required to understand that a particular drug is truly effective in the model, thereafter experimental approaches in humans will be more appropriate.
We have considered to develop an in vitro/ex vivo screen, after antibody mechanisms are identified in the epitope/cell function experiments, before using animals to assess compounds. For example, if we can find that (hypothetically) a novel cytokine is involved in mediating the downstream effect after antibody binding to a satellite glia cell in the DRG, and if effects of novel compounds to directly block the production of this novel cytokine are not well described in the literature, then instead of using animals it would appear more appropriate to develop either a primary cell line or if possible an immortalised cell line to first establish that a particular drug actually affects that pathway. *Therefore, for any identified mechanism that we will address with compound testing, we will carefully weigh whether the information available about the action of that compound is adequate to justify use in the model directly, or rather whether first assessment in cells should be evaluated* to clarify whether this pathway is actually sufficiently interfered with by the compound. Only the most efficacious drugs would then be tested in the animals.

Which non-animal alternatives did you consider for use in this project?

We did consider using human tissue either derived from biopsies, or from human tissue harvest after death in order to achieve part of our first aim, to identify epitopes. For clarity, in such research human tissues could be put in contact with the patients' autoantibodies. As autoantibodies 'bind' to tissue, it may be possible that such binding could be identified and from there the binding structures ('epitopes'), and mechanisms could be explored.
With regards to the use of non-animal alternatives to assess the effects of drugs, we will in part use nonanimal methods.

Why were they not suitable?

Human tissue: firstly our preliminary research has already indicated that the likely target tissues in humans are dorsal root ganglion cells, sub-groups of immune cells, or brain/spinal cord cells; these cells are not generally ethically accessible using biopsies in humans. Additionally, even if it was possible to take biopsies from these tissues, this process would not be useful to achieve our objective because it would not generate sufficient tissues to identify epitopes (see also below).
With respect to the use of tissue from human tissue banks, in the case of CRPS, binding is likely only possible if the patient has sustained a limb injury - we have tried previously to identify binding by using tissue from naïve mice (and checking whether the human antibodies bind to it) - this was not successful. Similarly it is hence unlikely that we would find binding in *human* tissue unless a distal limb injury has occurred shortly before tissue harvest - so that in order to have sufficient material we would likely require tissue from a large number of patients who died shortly following a distal limb injury, which is not feasible.
In the case of FMS, we will in parallel pursue an avenue of trying to identify staining with human IgG using suitable tissues derived from tissue banks (DRGs to start with). However, while this approach may eventually support the validation of animal research, it is not suitable for the identification of epitopes with currently available methods, because not enough human cell material would be available to make it possible to detect the molecular structures to which the antibodies bind.
Tissue from tissue banks is not alive and is therefore not suitable for the identification of cellular mechanisms.
Non animal alternatives to assess drugs: We will reduce the use of animals by first screening, where possibly, cell lines for compound effects after antibody exposure as described above. However the human organism is a complex system, and many molecular processes cannot be modelled in cells because many different cell types and sub-types are involved to produce the phenotype. This is particularly true for chronic pain. Therefore only using cells to assess compounds before human testing is not suitable.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Overview details about number of animals are given in the Action Plan section.
All numbers are maximal numbers. Animal numbers were discussed with an expert in statistics for rodent experiments. There are two protocols, A) Fibromyalgia, B) CRPS. Both protocols use the same approach: a mix of blood proteins ('immunoglobulin G antibodies' (IgG)) will be purified from the blood of patients suffering from either condition, or from healthy controls and will then be injected into groups of mice. Within that mix, some proteins are thought to cause the respective pain disorder.
Each of the two protocols has two components – component 1 relates to the objective of better understanding the mechanisms by which the proteins cause disease. No power calculation was considered appropriate for this section. We and others have conducted and published experiments before, outlining the typical number of animals needed. Component 2 relates to the objective of evaluating therapeutic substances.
component 1 – better understanding of disease mechanisms
Tissue binding studies will be conducted to understand to which tissues the human proteins bind – harvested tissues will be sliced very thinly; this will be followed by experiments investigating cell function.
To identify the molecular structures to which the IgG bind, methods will be refined in accordance with the cell type identified, and then sufficient mass of bound protein needs to be generated to allow analysis; this process is the central method to identify the exact binding; up to 10 animals need to be used to generate enough tissues for one experiment; note where the use of rats is required because use of mice is found to not provide sufficient

tissue, then fewer mice are needed; further where use of mice will provide sufficient tissue no rats are used. For all the above studies, we will in all cases first optimise the methods of preparing harvested cells by using primary cells not involving regulated procedures. We will only use mice that undergo regulated procedures as and when the preparation methods appear fully optimised. Grand total 1200 mice, 800 rats.
component 2 - compound testing
These experiments will be carried out only as and when the experiments above produce relevant information about molecular structures and mechanisms.
Testing of drugs (always mice) will generally be conducted by including 3 control groups, a healthy volunteer-IgG injected group that receives the therapeutic compound, plus disease- and healthy volunteer-IgG - injected groups that receive saline vehicle, i.e. generally 4 groups per compound will be tested; we pragmatically assume that we will need an average of 6 mice pre group. We intend to treat the animals in either the FMS- or the CRPS models for a maximum of 3 weeks, and we anticipate on average performing 1 repeat and one dose adaptation experiment/ compound. grand total n=1200 mice.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Randomisation and blinding, and limitation of the number of operators will reduce variability and animal numbers. Animal numbers and study design were discussed with an expert statistician and adapted - specifically it was confirmed that first pilot studies should be performed with each IgG preparation as appropriate and that for compound testing experimental numbers per group can be reduced to n=3 per group if outcomes are supportive.
Feedback was also received from an RRR specialist, and several design steps were streamlined reducing animal numbers, for example a previously planned step to elucidate epitopes for extreme FMS phenotypes was removed reducing the animal number needed by n=200; further the validity and strength of the mechanism in question for various compounds will now be tested first by first using cellular models and then taking forward the best-effective compound, where possible.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

- preservation of tissues in the staining experiments where appropriate, so that other tissues will be available at a later time, and mice are not just used for harvesting/assessing one single tissue type

- early switch from using mouse to rats for the epitope experiments, if needed, to avoid futile use of mice in cases where tissue harvest will be sub-optimal

- pilot studies for all compound testing experiments to elucidate effect sizes, power calculations then in communication with a statistician with experience in this field

- cellular studies to elucidate compounds with maximal efficacy

- use of non-protected species where this is possible in case of new compound testing not tried in rodents taking into account 3R toxicology advice

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare

costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

A) CRPS is a post injury condition and the pathogenicity of antibodies cannot be shown without setting an injury. We therefore need to use a model in which injury is induced, but our model is the most refined model available – it occurs immediately and is of short duration (right hind paw incision of plantar skin and plantar muscle) under general anaesthesia).

B) Transfer of human immunoglobulin (a blood protein) to rodents - the human antibodies are the core pathogenetic factor to be examined; we have refined our experiments for the FMS model by first examining both antibody binding and binding epitopes without using any model, instead using primary cells. Only if this does not render usable results will we transfer human immunoglobulin. Similarly, in the CRPS model in order to elucidate binding tissues and epitopes we will first try to avoid human immunoglobulin transfer all-together. This approach is the most refined approach as it minimises additional suffering from receiving the sensitivity-inducing human immunoglobulin antibodies

C) Similarly, when cell functions have been assessed in either the FMS or the CRPS models, we will attempt to move to the refined approaches of using either primary cells harvested without procedure (FMS) or cells harvested without human immunoglobulin transfer.

Why can’t you use animals that are less sentient?

We are using the lowest neurological model (mice) for the majority of our work. Rats only to be used when necessary.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Because we are interested in pain research any analgesics (outside the peri-operative period) are not appropriate but we will monitor the animals regularly (twice daily) including weight and any surgery will have a 7 day post-operative assessment as standard.
For the CRPS work, we will first use the Brennan model to identify tissue staining and epitopes, which does not need injection of IgG. Only where needed will we use additionally IgG injection.
All animals that will be involved in behavioural tests will be habituated to these tests to reduce any distress.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow “LASA best practice guidelines” for administration of substances and Home Office and LASA guidelines for aseptic techniques.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will check the NC3R website regularly. We are in regular contact to the responsible officers within the establishment's biological services unit and will enquire about advances (e.g. 3-6 monthly), and similarly have been offered support through the NC3Rs Regional Programme Manager and are grateful to take this up in a similar way.

Home Office

NON-TECHNICAL SUMMARY

112. Mechanisms of neuronal plasticity in psychiatric and neurodegenerative disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Schizophrenia, Alzheimer's disease

Animal types	Life stages
Mice	adult, juvenile, pregnant, embryo, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall objective of this project is to understand the molecular mechanisms of the brain's synaptic plasticity

(changes in the efficiency with which brain cells communicate with each other), and hence to facilitate the treatment of neurodegenerative and psychiatric diseases where synaptic plasticity is impaired. In particular, this project aims 1) to identify the signalling mechanisms involved in synaptic plasticity that are compromised in psychiatric or neurodegenerative disease; 2) to ascertain how dysfunction of these mechanisms affects neurochemical, functional and behavioural parameters relevant to psychiatric or neurodegenerative disease; and 3) to assess the efficacy of novel treatment strategies to ameliorate the disease-relevant changes.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Brain diseases represent an enormous and increasing burden on health care resources and inflict great suffering on patients and their carers. For example, Alzheimer's disease affects 10% of the population over 65, 33% of the population over 85 (WHO). The only currently available treatments delay deterioration, but are not effective in the long term, as they do not interfere with the neurodegenerative process. Total societal costs are estimated at £26 billion/year in the UK. Similarly, for schizophrenia, the lifetime risk in the general population is 1%, with the majority of patients affected for most of their lives, with an onset in late adolescence. Current drug treatments (dopamine antagonist anti-psychotic drugs) are effective against some symptoms, but not at all against others (and are associated with unpleasant side-effect profiles). Total societal costs were recently estimated at £12 billion/year. Hence novel pharmacological treatment strategies are urgently needed for these diseases.

What outputs do you think you will see at the end of this project?

The research will improve our understanding of the mechanisms allowing synaptic changes in the brain, and provide insight into how the processes go wrong in diseases such as Alzheimer's disease and schizophrenia. As a result, the research may also illuminate novel ways of alleviating these neurological diseases.

Who or what will benefit from these outputs, and how?

The short-term impact will be increased understanding of the biological basis of psychiatric and neurodegenerative disease. This will be evident as soon as the results are published, and will hopefully stimulate follow-on research in other laboratories. The longer-term output will hopefully be improved treatments for these diseases. Improved treatments will only be identified through rational drug development programmes based on an understanding of disease aetiology and testing of drug candidates in translational models. Our research will contribute to, and enable, this process.

How will you look to maximise the outputs of this work?

The intention would be to communicate the results of this project to as wide an audience as possible. Communication with the scientific community would be facilitated by peer reviewed publication of results in the highest-profile and widest-interest journals, as well as the usual specialist literature. We also have an extensive network of contacts in the pharmaceutical industry, and communication and collaboration with industry colleagues will be an important way to maximise the long-term output. An additional aim would be to disseminate results to the general public via open access lectures (e.g. during Brain Awareness week), articles in general, wide-distribution science periodicals, and also via Press interviews.

Species and numbers of animals expected to be used

- Mice: 4000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the only species used in this programme of research. Mice are widely-used to study the mechanisms underlying learning and memory. Their neuroanatomy is similar to that of humans, and behavioural tests are available which match closely with those used in humans. Animal use occurs only where it is not possible to test important hypotheses using in vitro systems.
The majority of studies use adult mice, since most psychiatric and neurodegenerative diseases affect adults. However, the onset of some very common psychiatric diseases such as schizophrenia, is during adolescence; hence juvenile mice are used on occasion. In addition, most of the environmental risk factors for schizophrenia operate prenatally or antenatally. Hence some studies will look at the effects of reproducing the environmental risk in pregnant females and in their embryos and neonates.
The use of genetically-modified mice is frequently the only viable strategy for studying the in vivo function of gene products which cannot be manipulated pharmacologically.

Typically, what will be done to an animal used in your project?

Mice, in some cases with genetic manipulations to elevate or reduce the level of expression of specific genes related to neurodegenerative or psychiatric disease, will typically receive injections of current drugs used to treat these diseases, or of potential future improved drug treatments. Often, mice will be identified by implantation of a subcutaneous microchip, so that non-invasive behavioural observation can be conducted.
Pregnant female mice may receive an immune mimetic agent to induce an immune response, in which case offspring will have been exposed to the maternal immune response prenatally.
In some cases, mice will receive intra-cerebral injection of an optogenetic or chemogenetic viral construct, or of a gene expression viral construct. In this case, the mice will later undergo surgery for electrophysiological recording under terminal anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

The abnormal behaviour that may be observed due to manipulation of a gene relevant to psychiatric disease, with or without environmental manipulation, is expected to be very subtle. Psychiatric disease-relevant phenotypes due to the manipulation of individual genes or genetic loci are invariably restricted to slight changes in the amount of locomotor activity, along with subtle cognitive and affective changes that can only be detected using sophisticated techniques. Neurodegenerative disease relevant phenotypes due to the manipulation of individual genes or genetic loci are also relatively mild, with behavioural change typically limited to small reductions in cognitive abilities.
Pregnant female mice are expected to experience weight loss due to the immune response following injection of immune mimetics. However, this is expected to be transient, with mice rapidly recovering their initial weight. No

more adverse effects such as abortion, post-natal health issues or maternal rejection are anticipated.
The drugs used are expected to produce only subtle pharmacological effects. Some transient minor discomfort due to injection is expected.
The surgery involved in intra-cerebral injections will produce post-operative pain, which will be mitigated with analgesics. Rarely there may be post-operative wound infection, which will be treated with antibiotics.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice - mild severities anticipated in 81% of animals, moderate severities in 18% of animals, 1% of animals are non-recovery.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Cell cultures have limitations in modelling the complex network nature of the CNS, and there inevitably comes a stage where the theories obtained from the cultured cells have to be tested in vivo. To study the function of the novel genes we have identified, we have felt that the information to be gained justifies the use of genetically-modified mice, although we still favour the use of in vitro and cell culture studies wherever possible. Moreover, whilst cellular level assays are used to test whether pharmacological and genetic interventions can rectify pathology at the single cell level, it is only by testing these at the whole brain/organismal level that one can truly assess whether these translate to improvements in paradigms reflecting cognitive function.

Which non-animal alternatives did you consider for use in this project?

We have been able to develop cell culture models which allow us to pursue many aspects of our studies in culture rather than in the whole animal. This involves the use of immortalised cell lines in many cases, and also the use of primary neuronal culture prepared from mouse tissue. We have taken the opportunity to divert most of our studies into the cell culture system. We are optimistic that we will be able to continue studying the molecular basis of neurological disease mainly in cultured cells.

Why were they not suitable?

Cell cultures have limitations in modelling the complex network nature of the CNS, and the subtle and multi-faceted dysfunction in these networks that underlies psychiatric and neurodegenerative disease. There inevitably comes a stage where the theories obtained from the cultured cells have to be tested in vivo.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken

to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The proposed experimental designs and methods of analysis of the results have been optimised over a number of years, and discussed with statisticians where appropriate. Many of the mice are involved in breeding programmes, and we have extensive experience in optimising breeding strategies to minimise the numbers of mice involved.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Much of our research is conducted using ex vivo materials (electrophysiological, biochemical and morphological assays and genetic and pharmacological interventions). Furthermore, the use of cultured systems (organotypic and dissociated cell culture) enables the preparation of multiple cell based assay systems from a more limited number of animals.
Since much of our work utilises either ex vivo neurochemical and genomic investigations of tissue samples, or primary culture approaches, only a minor proportion of animals generated by breeding are then studied in subsequent protocols.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use efficient breeding strategies, so that generation of surplus mice is avoided wherever possible. We will obtain tissue from mice used in behavioural studies, rather than generating separate groups of mice for tissue collection. We will use pilot studies employing minimal numbers of subjects, where poorly characterised drugs are being used., and we will use within-subjects designs for drug studies in general, to maximise statistical power and reduce numbers of subjects used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are widely-used to study the mechanisms underlying learning and memory. We will use genetically-modified mice, as this is frequently the only viable strategy for studying the in vivo function of gene products which cannot be manipulated pharmacologically. However, manipulation of the genes associated with psychiatric and neurodegenerative disease in general produces only very subtle phenotypes (we have studied more than 20 of these genes in transgenic and knockout strains). Genetic manipulation of these genes in mice is therefore not expected to produce overt pain, suffering, distress or lasting harm.
We will also study the responses of mice to the administration of drugs/agents expected to affect plasticity processes. The agents that will be administered are also generally well-characterised in other contexts, and are not expected to produce overt pain, suffering, distress or lasting harm.

We will also study the effect of reproducing environmental risk factors for psychiatric disease. In humans, and also in mice, exposures to these factors have no lasting effects on their own, and, when combined with genetic risk factors, produce a subtle enhancement of the effect of the mutation. No overt pain, suffering, distress or lasting harm is anticipated.
In some cases, where there are no alternative means to manipulate the CNS expression of these genes, we will also inject agents to affect local gene expression in the CNS. Pain and discomfort due to surgery will be controlled with analgesics, and subsequent studies will be performed under terminal anaesthesia.

Why can’t you use animals that are less sentient?

Psychiatric and neurodegenerative diseases are, in general, not apparent in humans until the CNS is mature. In many cases, increasing age is a risk factor. The use of immature life stages is therefore not appropriate for this work. While the mouse CNS is similar anatomically and neurochemically to the human CNS, and mice have a behavioural repertoire with clear parallels to human behaviour, the same is not the case for lower species. hence mice represent the minimum species that can be studies.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will monitor new strains of mice very carefully to assess their phenotypes. Post-operative care will include close monitoring and the use of analgesics.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the ARRIVE guidelines throughout.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continue to monitor advances in the 3Rs, and look for opportunities to improve our research strategies.

Home Office

NON-TECHNICAL SUMMARY

113. Mechanisms of seasonal rhythms in physiology and behaviour

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

chronobiology, neuroendocrine, genomics, epigenetics, behaviour

Animal types	Life stages
Siberian hamster	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To identify the genes involved in timing seasonal physiology and immune function. Understanding predictable oscillations in seasonal genes will help inform management and welfare of human and domestic animals.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

All animals, including humans, show long-term rhythms in health and disease. For example, acute and chronic illnesses (e.g. seasonal affective disorder) have annual peaks and declines in incidence and severity of pathology. Our understanding of predictable, long-term rhythms at the genetic, molecular and cellular signalling level, and across internal organs is poorly developed. By using well characterized day length manipulations, the work can easily replicate robust seasonal changes in physiology and immune function by simply placing animals in rooms that mimic summer-like long days and winter-like short days. The work described in the licence will help us uncover the genome plasticity that underlies long-term changes in physiology, immunology and behaviour.

What outputs do you think you will see at the end of this project?

The primary benefit of the work is the advancement of scientific knowledge on the role of seasonal genes and hormones for the control of long-term changes in physiology, behaviour and immunity. The knowledge gained will be directly relevant for human and animal health and welfare. By discovering new ways in which genes are in control, the work might help identify new light mediated approaches for human and animal care. It is anticipated that 10-15 publications will be achieved.

Who or what will benefit from these outputs, and how?

The short-term benefits of the outputs will be for academics interested in how long-term rhythms (e.g. seasonal) in physiology, behaviour and immunity are controlled. In the long-term, medical and veterinary practices will be interested in which genes and hormones are involved in timing seasonal changes in physiology and immune function.

How will you look to maximise the outputs of this work?

Outputs of the work will be maximised by dissemination via publication in open access and international sources. The findings will also be presented at public engagement events and communicated to the national media. Real-time updates on research progress are readily provided on social-media. The training provided will facilitate the development of the next generation of cutting-edge scientists and communicators.

Species and numbers of animals expected to be used

- Other birds: No answer provided
- : 1010

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Hamsters and quail models have been valuable examples of mammalian and avian species due to the well characterized annual change in reproduction, energy metabolism and immunity. Only hamsters and quail have robust, reliable and repeatable changes in seasonal physiology in response to a simple change in laboratory light conditions. Moreover, these animal models benefit from over 50 years of scientific literature, provided a solid basis to advance our scientific understanding of the genetic of seasonal rhythms. The combination of breadth in scientific knowledge and robust seasonal physiology in laboratory conditions make hamsters and quail the ideal animal models.

Typically, what will be done to an animal used in your project?

Most animals will experience manipulated changes in day length as a cues to mimic seasonal rhythms in physiology. To better understand how hormones and genes control seasonal rhythms in physiology, some animals may receive injections of melatonin, thyroid hormones, gonadal steroids or prolactin. In few cases, some animals may experience surgical procedures that will allow the function of precise seasonal genes to be controlled. Surgical procedures will be limited to a single brain injection into the middle of target brain regions involved in timing seasonal rhythms. Animals will only experience one procedure and the duration is never longer than 32 weeks.

What are the expected impacts and/or adverse effects for the animals during your project?

There are few to no expected adverse effects for the animals during the project. All studies will use manipulated changes in day length to induce seasonal changes in hormone concentrations. In the few instances, some animals may show weight loss, infertility and reduced immunity that parallels what the animal would experience in the wild. Pain and suffering is minimised by good surgical and aseptic techniques, suitable anaesthesia, good peri-operative care and adequate provision of pain relief. Any animal showing any signs of ill health will be closely monitored, receive veterinary treatment or will be humanely euthanized.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severities range from mild to moderate. The approximate proportion of animals are for mild (66%) and moderate (34%).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are the only model for the proposed licence because it is not possible to study the brain using cultured cells or computer modelling. At present, to understand how external factors such as light affect behaviour, the brain, and immune interactions requires the use of live animals. The research programme seeks to identify means to replace gene-behaviour studies by incorporating mathematical modelling when available.

Which non-animal alternatives did you consider for use in this project?

Unfortunately, non-animal alternatives do not exist at the moment. To understand how the brain detects and integrates external information and internal hormonal signals requires the use of live animals. The only potential alternative to consider for the licence would include artificial intelligence.

Why were they not suitable?

The use of artificial intelligence is limited due to the lack of knowledge of how the brain times long-term rhythms at a genome, molecular, and physiological level. It will likely be decades before such artificial intelligence can be used. Artificial intelligence would be welcomed because of the replacement in animal usage.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Estimation of numbers of animals used are determined from several sources. The sample sizes for each protocol are derived in part, from decades of published literature that use hamster and quail models. These numbers are also shaped due to several years' experience working with these models.
Lastly, statistical models are employed to limit the number of animals used for each protocol.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Tests that are regularly conducted to assess statistical significance and power include multi-factorial ANOVAs and Repeated measures ANOVAs. Appropriate advice is taken from qualified statisticians. Sample sizes are based on statistical power analysis from several prior experiments and power calculations conducted with a statistician. When power values are observed to be consistently high (>0.8); sample sizes will be reduced to include the minimum number of animals necessary. Built into the experimental design and dissemination of the results are the ARRIVE guidelines established by the
NC3Rs.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To reduce the use of animals, all tissues are collected at the end of the experiment and shared with colleagues or archived for future use. By engaging in the sharing of tissue we reduce the animals used for research.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Hamsters and quail are the best species to study seasonal rhythms in physiology, behaviour and immune function. There is a large scientific literature on this species and alternative models (for example mice and chicken) do not exhibit the large brain, physiological and immune changes over long-term, seasonal time scales. The methods used will allows the identity of genes that generate seasonal rhythms to be characterized and discovery how sex steroids, melatonin, thyroid and prolactin hormones regulate the timing of gene expression. The methods used, such as hormone injections or removal are the best suited to minimize harm while allowing the role of hormone action to be uncovered.

Why can't you use animals that are less sentient?

Unfortunately, to uncover the genetic and physiological signals that provide long-term timing requires adult animals. Moreover, only Japanese quail and Siberian hamster show robust, reliable and repeatable changes in

seasonal physiology and are supported with scientific literature that span 50 years of research. Common biomedical and domestic animals have a significantly reduced seasonal rhythm and are not optimal to study the objectives of the research programme.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinements for injections include good technique (e.g. rotation of injection site, single use of needles, sympathetic handling) and the minimization of the number of injections (never more than daily for 6 weeks). In terms of surgery, pain and suffering is minimised by good surgical and aseptic techniques, suitable anaesthesia, good peri-operative care and adequate provision of pain relief. Pilot studies that use alternative procedures (e.g. apple bits, pellets) will be used to optimize as these potentially new procedures may result in minimal or complete elimination of current approaches.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The National Centre for the Replacement, Refinement and Reduction of animals in research website is readily checked. The resources available, such as literature on experimental design and ARRIVE guidelines are reviewed and incorporated into experiments to maintain the most refined procedures are conducted.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The scientific literature is continually reviewed and veterinarians consulted for alternative surgical treatments and novel means to alleviate adverse effects. In addition, we will keep abreast of local guidelines developed by our local AWERB and seek their advice where appropriate. To prevent duplication of experimentation and facilitate sharing of tissue material, scientific conferences are attended and discussion held with colleagues.

Home Office

NON-TECHNICAL SUMMARY

114. Mechanisms of structural and functional plasticity in cortex

Project duration

5 years 0 months
Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Mice	adult, juvenile, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim is to understand the mechanisms of functional plasticity and structural plasticity in the healthy and diseased brain.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

By understanding which behavioural, sensory deprivation and activity paradigms induce or prevent plasticity, we can begin to understand potential mechanisms that are critical for the initiation and maintenance of synaptic plasticity, or the changes associated with memory formation and learning. The experiments outlined here are an important first step in determining the mechanisms of plasticity, which will be critical in the future development of treatments for a number of neurological disorders that have disrupted plasticity.

What outputs do you think you will see at the end of this project?

At the end of this license, we expect to have several publications on the mechanisms of plasticity as they relate to behavioural paradigms, spatial scales, and cell type specificity. We expect to have collaborative publications on plasticity mechanisms in disease states, particularly Alzheimer's disease.

Who or what will benefit from these outputs, and how?

By understanding the mechanisms of plasticity, we can begin to understand the conditions that are critical for the initiation and maintenance of plasticity. Then, using animal models for diseases, we can determine which of these mechanisms are faulty in the disease states and may serve as potential targets for future treatments. We expect that in the next five years, we will have 1) established a list of important plasticity mechanisms that can be tested to determine if they are perturbed in disease states, 2) established the disease models, particularly for Alzheimer's disease and 3) tested the degree to which some of the synaptic plasticity mechanisms are deficient in these disease states. Any important plasticity mechanisms that we find in healthy animals, will then be tested by ourselves or our collaborators to determine if they are perturbed in disease states. We have collaborations with scientists based in pharmaceutical companies and as we find plasticity mechanisms that are perturbed in the disease state, we will work in collaboration with these colleagues to establish potential treatments that target these plasticity mechanisms.

How will you look to maximise the outputs of this work?

We have several collaborations with scientists who are working on diseases of plasticity around the world, and we will share data, analysis tools and resources between these laboratories. We are a part of two large worldwide consortia for learning and plasticity, which involve sharing data and analysis tools between dozens of laboratories. This will reduce the number of replicated experiments in the field and the number of experiments that we need to do in our laboratory. All of our findings are made public with open access and our analysis tools are freely shared on public forums. We will give public talks about our new findings related to plasticity and diseases and work with public partners to make this new knowledge known.

Species and numbers of animals expected to be used

- Mice: 2000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will do these experiments in mice because of the number of available mice that are transgenically altered

and the comparison with previous studies in the scientific literature. Using transgenic mice has the distinct advantage that we can use mice with the phenotypes for diseases, such as Alzheimer's disease, Parkinson's disease and epilepsy that have disrupted brain plasticity. We will use adult mice, since these experiments are most viable in adult mice and the diseases of plasticity are largely associated with ageing.

Typically, what will be done to an animal used in your project?

Typically, a transgenic animal will undergo a surgery, where a small part of their skull is replaced with a glass coverslip to give us repeated optical access to the brain. They will then have four weeks to recover from that surgery. After that time, they will have a number of non-painful behavioural tests, some of which will also involve measuring from the neurons in their brain using light. These tests may be repeated several times over the duration of typically up to one month. Part way through the tests, the animals could either learn a new task or undergo sensory changes (like being in the dark) and we will measure the neurons' changing responses to the changing environment. At the end of these behavioural measurements, the animals will be humanely sacrificed and their brain tissue will be used for post-hoc histological or electrophysiological measurements.

What are the expected impacts and/or adverse effects for the animals during your project?

The animals will undergo surgery of moderate severity. This means that the mice will be quiet and move less for a day or two after surgery. The animals will be given multiple types of pain relief after surgery. Animals might lose up to 15% of weight, but will typically lose 5% or less and animals will regain weight loss within two to three days. In the event of infection or at the end of the procedures, the animals will be humanely sacrificed in consultation with the veterinary staff.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately 1000 mice will undergo a surgery of moderate severity, which means that they will have a one or two surgeries. Approximately 300 mice will undergo non-recovery procedures, where they are given terminal anaesthesia at the beginning of the procedure. Approximately 2000 mice will be bred transgenically, which is a mild severity. These animals will then enter one of the other two protocols.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our objective is to characterise the mechanisms of plasticity in the healthy and diseased brain. The field of in vivo plasticity is relatively new and the current understanding of basic mechanisms of plasticity is poor. Furthermore, many of the disease models have only recently been developed and basic characterisations are still underway. Thus, there are currently too many unknowns to use exclusively computer models to determine the expected mechanisms of structural plasticity in either the diseased or healthy brain.

Which non-animal alternatives did you consider for use in this project?

We have considered both computational models and human plasticity studies. We will work with computational collaborators on models to test potential hypotheses and limit the potential outcomes, which will help us with our

experimental design and limit the number of animals necessary. We also have collaborators working on human plasticity and will work closely with them to design mechanistic animal experiments in a way that will be most quickly and directly beneficial to human health.

Why were they not suitable?

As stated above, there are currently too many unknowns to use exclusively computer models to determine plasticity mechanisms. For human experiments, we cannot measure the mechanisms of plasticity without animal results first.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated these numbers using a power analysis of group size for each set of experiments we have planned. We estimated effect size based on our pilot experiments, past work and expected effect sizes from the literature.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In our experimental design, we always collect as much data as possible from each animal. With longitudinal experiments, we repeatedly measure the same synaptic structures, synaptic activity or cellular activity over a long period of weeks. This approach allows us to directly compare the structural/functional plasticity in the same synapses, cells and animal before and after sensory deprivation or alterations in behaviour. Furthermore, in each animal, we can collect data from hundreds of synapses and dozens of cells, increasing the amount of data from each animal used and reducing the overall animal number necessary. Additionally, our experiments are designed over the same time course for all paradigms, thus we will use shared controls whenever scientifically feasible, to reduce the number of animals used in total.

The proposed experiments will generally use factorial experimental design, which will maximise the data collected from each animal. We will use the appropriate statistical methods for analysing the data and seek statistical advice as necessary from the Bio statistics group at our university.

We have also made methodological advances, based on technologies that have emerged in our field, which now allow us to collect more detailed data using the same surgical approach. Specifically, we are able to measure the functional properties of synapses, not just their structural changes levels over time. This helps reduce the variability that arises in our data and means that we need fewer overall experiments. This is reflected in our overall animal numbers, which are fewer than half of the total number of animals on our last project license application.

In all experiments, we will use the following guidelines to ensure that data collected from our animals is viable:

1. All surgeries will be performed in aseptic conditions in a designated surgery space with SEP status

2. Groups will be randomised and blinded

3. All data analysis will be done blindly

4. To reduce the effects of inflammation following surgeries on experimental results, we will give the animal sufficient time to recover from surgeries (typically 4 weeks).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will test hypotheses using computational models in order to refine the parameter space of the experiments before starting animal experiments and thereby reduce the number of necessary animal experiments. We always do pilot studies with a few animals to ensure the correct time course and experimental paradigms for our experimental purposes before larger scale studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Choice of species:

We will do these experiments in mice because of the number of transgenic mouse lines available. Using transgenic mice has the distinct advantage that we can use mice with the phenotypes for diseases, such as Alzheimer's disease, Parkinson's disease, and epilepsy, which have disrupted plasticity. These mouse lines allow us to test potential mechanisms of plasticity and their role in these diseases.

Choice of method/minimise suffering:

By using chronic imaging in combination with cranial window surgery preparations, we can follow the same synaptic structures or cells over period months. This preparation is ideal for examining plasticity, because it allows us to examine how the same synapses are changing their structure and function over time. Because changes in plasticity in response to sensory changes can take up to several days to occur, addressing these questions in acute slices or slice culture is not possible. Additionally, to examine the role of plasticity in behaviour, we need to do these experiments in intact animals that are capable of learning and behaving. We are committed to refining the protocols and in the past five years have made many refinements to our methods to reduce the number of animals necessary (and as a result, have more than halved the number of animals in our project license). For example, we have used technological advances in the field to increase the yield of neurons recorded from 10s per animal to 100s and 1000s per animal, and to use viruses and transgenic animals, rather than in utero electroporation, which reduces the number of animals necessary and decreases the complications from the procedure. We have developed new methodological approaches that allow us to measure the functional responses from many synapses over many days, where previous work was only able to measure at a single time point. This approach allows for within animal comparisons and reduces the number of animals necessary in total, as well as increases the amount of data collected per animal without any further painful procedures. Past work that records simply the presence and absence of synapses (rather than their functional responses), requires far more data per study, since averages are across a large number of synapses with different properties. By being able to sort the synapses by their response property, we are able to see effects with far fewer animals.

Furthermore, by collecting behavioural data (novel object recognition, open field, foraging and nesting behaviours) in our mice that have undergone imaging, at a later date when we move into studying disease models of plasticity pathology, we can test rescue approaches on the known affected behaviours to determine if plasticity rescues have worked without surgical interventions necessary for chronic imaging. We can then focus our in vivo imaging experiments on rescues that have a behavioural effect.

By using chronic imaging, we can make comparisons within an animal, which increases our statistical power and allows us to use many fewer animals. Furthermore, this protocol minimises suffering, as the initial surgery is the only one in which any pain may occur. All imaging sessions afterwards will only require either light anaesthesia or no anaesthesia.

In addition to chronic imaging, we plan to prepare acute slices from mice. These terminal experiments will take place entirely under anaesthesia and therefore will have minimal suffering. We plan to use these experiments to test a number of potential mechanisms before testing them in vivo, which will help reduce the number of animals undergoing the in vivo paradigm and that may suffer as a result.

Why can’t you use animals that are less sentient?

A number of our experiments are done in animals that are terminally anaesthetised, but in order to look at behavioural outputs, the animals must be awake and behaving. Our recent work has shown that plasticity mechanisms in the intact brain are not the same as those measured in reduced preparations, therefore, we must do some of our experiments in an intact system. Animals that are at a more immature life stage are unable to perform the behaviours associated with plasticity and there are major differences between plasticity in young and older animals. Given that many plasticity diseases are associated with ageing, we need to work in adult animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have made many refinements since our last project license. We use refined holding techniques for the animals, as well as group housing and enrichment. We use post-operative analgesics and will refine these with advice from the NVS to ensure that we are using the best possible option given our experiments. The animals will be given multi-modal peri-operative analgesics and allowed to recover for typically four weeks after surgery before undergoing any further procedures. We have also moved to not using anaesthesia during our imaging experiments. A majority of our repeated imaging experiments (>75%) are done in awake animals.

We have also refined our sensory deprivation approaches and always use the intervention that requires the least surgical intervention.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We are constantly reading the most current advice on refinement in the literature, which is brought to our attention by our local animal staff. We have local holding for the animals, and thus they are housed with littermates and with enrichment. We will also use refined animal holding techniques, specifically tube handling. We will also consult recommendations on the ARRIVE and NC3Rs websites, regarding animal welfare. Regarding refined injections of substances, we will consult the LASA guidelines and recommendations from the Working Group, together with published guidelines for substance volumes and routes of injection.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in the 3Rs from several sources. We are in regular contact with our animal care staff, as they come directly into our laboratories to care for the animals daily. They, together with our NAWCO, make suggestions for 3Rs refinements and develop approaches to implement them in our animal holding area. Additionally, since our animal area is a part of our laboratory, we are able to locally implement any 3Rs advances immediately, without consultation from other laboratories. We also have a 3Rs Regional Manager

that works with our animal staff, with whom we will consult and annual meetings that highlight the 3Rs advances. We will also view the NC3R's Website periodically.

Home Office

NON-TECHNICAL SUMMARY

115. Mechanisms underlying circadian and visual responses to light

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Circadian rhythms, Vision, Light, Optogenetics, Sleep

Animal types	Life stages
Mice	adult, juvenile, neonate, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Light detected by the retina plays a key role in setting our circadian clock to the external environment, as well as regulating sleep and alertness. This project will characterise the photoreceptors driving these responses, determine how our environment may influence them and use our understanding of these mechanisms to treat retinal disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Light is detected by the mammalian retina by specialised cells called photoreceptors. However, the photoreceptors mediating visual and circadian responses to light are different. Work on circadian responses to light has identified an additional retinal photoreceptor system, based on a blue-light sensitive protein called melanopsin. As a result, light which is suitable for vision may not be ideal for our circadian physiology. The circadian effects of light are of increasing concern in our modern 24/7 society as the majority of the populace are exposed to artificial light at an inappropriate time of day. This includes artificial lighting in the work and home environment, as well as from light-emitting devices such as smartphones, tablets and computers. In addition, ~20% of the workforce perform work at night, on fixed, rotating, or irregular schedules where irregular or inappropriate light exposure is unavoidable. Aberrant light exposure has the potential to disrupt our circadian rhythms and sleep. Long-term circadian rhythm and sleep disruption has been linked with a wide range of adverse health outcomes, including impaired concentration and performance, mood disturbances, metabolic disease, cardiovascular and neurological disorders and even cancer. Understanding the underlying biology is therefore essential if we are to ensure that our light exposure is optimum for both circadian and visual function. In addition, ~20% of the US and European workforce perform work at night, on fixed, rotating, or irregular schedules Approximately 20% of the US and European workforce perform work at night, on fixed, rotating, or irregular schedules.

As well as responding to changes in the light environment, many organisms such as flies and birds are also sensitive to magnetic fields which they use for orientation and migration. Recent work has shown that the mammalian retina may respond to magnetic fields, influencing both circadian and visual responses. Given the prevalence of man-made magnetic fields - for example from power lines and mobile networks - understanding how they affect our physiology is clearly important.

Finally, understanding the mechanisms of light signalling provides opportunities for treating vision loss. Expressing light-sensitive proteins is sufficient to make neurons respond to light - a method termed optogenetics. Using optogenetics in cells which survive retinal degeneration provides a promising avenue for restoring visual function. The circadian photopigment melanopsin has been shown to provide an ideal candidate for such optogenetic approaches to visual restoration.

What outputs do you think you will see at the end of this project?

A primary outcome of this project will be an increased understanding of the contribution of different retinal photoreceptors (rods, cones and melanopsin pRGCs) to circadian and visual responses. This will particularly focus on regulation of circadian rhythms, sleep and alertness, but may also benefit many other aspects of physiology. As these photoreceptors differ in their sensitivity to the intensity, colour and timing of light exposure, this will enable us to design lighting that is optimal for both circadian health and visual function. This

fundamental scientific knowledge will provide the basis for future studies in human subjects to test the efficacy of such interventions. In this way, the development of optimised lighting strategies also has potential commercial application in circadian effective lighting. This will benefit the general populace and will be of particular relevance to shift-workers. The identification of new strategies to improve circadian lighting are expected to occur within the timeframe of this project.
Secondly, work on the role of the retina in magnetoreception will provide a mechanistic understanding regarding how magnetic fields influence retinal signalling in mammals. Whilst responses to magnetic fields have been widely studied in other animals, evidence from mammals is critical if these findings are to be relevant to humans. Moreover, such data will enable the power of mammalian genetics to be used to study the precise molecular and cellular mechanisms involved in magnetoreception. A key output of this work will be the necessary data to allow testing in humans to determine if environmental magnetic fields influence our visual or circadian physiology.
Finally, work on optimising retinal optogenetics provides an essential step in improving treatments of human retinal disease. Optogenetic treatments are already undergoing clinical trials. However, improving the cell-specificity and timing of these treatments has the potential to improve the degree of visual restoration achieved. Such data may provide the basis of subsequent clinical trials on optogenetic therapy.
At all stages, outputs will be published in peer-reviewed journals. Key outputs of this work will be in improving existing therapeutics - in particular improved light and optogenetics. These outcomes are likely to occur within the timeframe of this project. However, understanding the signalling pathways underlying these responses also has the potential to identify new targets for regulating circadian rhythms (for example in jet-lag or shift work). Development of these targets to new treatments is likely to occur beyond the timeframe of this project.
Translational work in humans will be conducted in collaboration with other research groups with expertise in human circadian and visual research, particularly in the case of clinical trials for optogenetics.

Who or what will benefit from these outputs, and how?

The use of artificial lighting is prevalent throughout the world - in both home and workplace lighting as well as from light-emitting electronic devices. With the increasing use of LEDs, light is now cheap and be easily optimised in terms of intensity, colour and timing of exposure. This work therefore has the potential to benefit a large proportion of the populace due to the widespread use of artificial lighting. In the short-term, over the course of this project, many basic scientific questions relating to the optimisation of circadian lighting can be addressed. For example, this will include optimising the intensity and colour of evening lighting to minimise circadian responses. These findings will then be translated to human studies before dissemination to lighting specialists and the wider public in the mid- to long-term.
Whilst the responses of animals to magnetic fields has attracted interest from biologist for decades, evidence for effects in mammals has always been lacking. This is of particular concern as our modern built environment contains numerous sources of magnetic fields, which have the potential to affect our physiology and health. Again, outputs from this project will provide key mechanistic data on the biological processes that are affected by magnetic fields, which are essential for properly designed human studies. Key mechanistic data is likely to be obtained within the timeframe of this project, and will form the basis of human studies that may occur beyond this project.
Finally, due to increasing lifespan, visual loss from retinal disease affects an increasing proportion of the populace. For example, in the UK age-related macular degeneration (AMD) affects 5% of those over 65 years of age and inherited retinal diseases such as retinitis pigmentosa affects 1 in 4000 people. Such vision loss can be irreversible. Optogenetic strategies provide a promising approach to treating retinal disorders, restoring light sensitivity and visual function. Internationally, clinical trials using optogenetics for retinal disease are already underway. Whilst data obtained within the timeframe of this project will benefit the design of future clinical trials, the clinical translation of this research is expected to occur beyond the timeframe of this project.

How will you look to maximise the outputs of this work?

We have extensive collaborations with other groups at our establishment, throughout the UK as well as internationally. This includes collaborative funding as well as acting in an advisory capacity - particularly in the

areas of light and circadian rhythms. As well as primary scientific publications, my lab has a strong track record in the development and dissemination of new methods. This has included non-invasive methods for measuring sleep, circadian rhythms, pupil responses and body temperature, as well as resources for measuring the effects of light and using existing genomic data in metaanalyses. As part of this work, we routinely make all published data accessible to other groups. I have also been involved in specialist training schools and developing online resources to help disseminate expertise and help others use the methods we have developed.
In addition, our group has a strong track record in public engagement. Our research was featured at a major museum exhibition and has been the basis of animated videos explaining the importance of circadian rhythms. We plan to continue such outreach work as part of this project to maximise the outcome of this project.

Species and numbers of animals expected to be used

- Mice: 20,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will exclusively use mouse models. Mice have well studied circadian and visual systems which have been shown to be highly relevant to humans. The availability of extensive genetic and transgenic resources provide ideal tools for studying the mechanisms underlying these aspects of physiology and behaviour. The majority of studies will be conducted in adult animals, though tissue may occasionally be collected from neonate or juvenile mice.

Typically, what will be done to an animal used in your project?

The majority of animals used in this project (75% of all animals) will be in the breeding of specific genetically altered mice for subsequent tissue collection or testing visual or circadian responses.
Subsequent testing may involve:

- Circadian testing (~15% of all animals). A typical experiment will involve housing genetically altered and control animals under different environmental lighting conditions over an extended period (typically 2-8 weeks), whilst monitoring voluntary home cage activity via cameras, sensors or voluntary wheel-running. During this time, animals may be exposed to light of different intensities or colours, or low frequency magnetic fields.

- Visual testing (~5% of all animals). This typically involves a battery of simple behavioural tests involving brief restraint to measure pupil responses or exposure to a novel environment to measure visual function. Animals may be briefly anaesthetised to study electrical responses from the surface of the eye or to image inside the eye.

- Behavioural testing (~5% of all animals). This will include exposure to a novel environment to study learning and memory or anxiety. Occasionally, tasks may require food restriction so that food can be used as a reward.

- Administration of substances (~10% of all animals). Substances (drugs or viral vectors) may be administered to modify visual or circadian responses. This may involve systemic injections as well as targeted injections to the eye. More rarely (~1% of all animals), stereotaxic injections to the brain may be

required. Implanted devices to allow the controlled release of substances may also be used (~2% of all animals). These animals will then undergo circadian, visual or behavioural testing.

- Implantation of devices. In a small proportion of animals (~1% of all animals), a device may be implanted to measure body temperature, heart rate (electrocardiography) or brain activity (electroencephalography).

NB: As specific phenotyping screens are not mutually exclusive, the proportions provided will not total 100%.

What are the expected impacts and/or adverse effects for the animals during your project?

A common adverse effect will be impaired visual or circadian function. This may involve using mice which are blind or have impaired circadian rhythms and sleep. Blindness in laboratory mice is not associated with any additional health effects, as they use their other senses - particularly smell - to navigate their home environment. However, circadian defects may result in changes in metabolism or behaviour. This may involve increased or decreased weight, as well as changes in the level and distribution of normal activity. Rarely, changes in social behaviour may also occur.
Circadian and visual testing are non-invasive, but may be stressful due to exposure to an altered environment. Some forms of visual testing may require repeated brief anaesthesia. Appetitively motivated behaviour may require food restriction, which will result in weight loss. Administration of substances may involve transient pain at the injection site. Where intraocular injections are used, brief anaesthesia is required, though animals rarely show signs of ocular irritation. Stereotaxic injections to the brain and implantation of devices require surgical interventions that are associated with pain during the recovery period, and a small chance of wound breakdown or infection.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Breeding (75% of all animals). Mild 20%, Moderate 55%.
Circadian and visual testing (15% of all animals). Mild 5%, Moderate 10%.
Administration of substances (9% of all animals). Mild 5%, Moderate 4%.
Telemetry (1% of all animals). Moderate 1%.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This work involves the study of the mechanisms underlying complex physiology and behaviour. This includes neural, hormonal and behavioural mechanisms, all of which interact. As such, no alternatives to animal models are available.

Which non-animal alternatives did you consider for use in this project?

We have considered the use of in vitro cellular models, mathematical models as well as the use of human

participants and patient groups. Whilst basic cellular processes, such as molecular rhythms and light responses can be studied in cellular models in vitro, these cannot recapitulate the full range of physiological processes that occur in vivo - particularly with regard to whole organism behaviour. Similarly, mathematical models allow key variables to be studied in detail, but are dependent upon detailed in vivo data. Predictions from mathematical models also typically require validation in vivo. Finally, human studies enable the translation of basic science data, but are not suitable for detailed mechanistic studies on the genes and pathways underlying these responses. However, the alternatives described above will be used in addition to the animal studies described in this project.

Why were they not suitable?

The alternatives described above are not suitable for studying the complex mechanisms underlying visual and circadian light inputs and the regulation of behaviour. Whilst we still use such approaches in conjunction with the work described in this project, the study of in vivo animal physiology and behaviour is essential.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals used is based upon extensive previous experience of similar work over the last 20 years. For the breeding and maintenance of transgenic animals, we would maintain up to 10 different genetically altered lines. Each line typically results in the generation of ~200 mice/year, or 1000 mice over the 5 year project. However, with the use of conditional and inducible transgenics with multiple alleles, more animals may need be bred to obtain the necessary genotypes (~400 mice/year or 2000 over the 5 year project). We would expect to maintain 5 knockout/mutant lines (1000 mice/year or 5000 over the 5 year project) and 5 conditional lines (2000 mice/year or 10000 mice over the course of the project). This gives a total of 15000 mice over the course of the project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To reduce the number of animals used during the course of this project, we maintain a close oversight of all GA lines and plan and sign off all experiments in advance to ensure that appropriate sample sizes are used to optimise statistical power. As well as the NC3Rs Experimental Design Assistant, we routinely use G*Power for sample size and power calculations. We have weekly meetings with all PILs at which planned and ongoing experiments are discussed. These meetings also reduce the number of animals used by allowing multiple questions to be addressed simultaneously as well as optimising tissue harvesting.
Where certain allele combinations are required, we calculate the necessary breeding strategies well in advance. This planning phase may result in some experiments not proceeding where the number of animals required to produce the necessary genotype is inefficient. We routinely cryopreserve lines to minimise the need for maintenance breeding of lines that are not in active use.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We practice efficient breeding and routinely archive our GA lines when not required to minimise unnecessary breeding. Where data is not available in the literature to determine appropriate sample sizes, we conduct pilot studies. Where possible we also use within group experimental design and counterbalancing to increase statistical power and therefore reduce necessary sample sizes. We also routinely use mathematical modelling to determine the optimum distribution of sample sizes across different time points. We also take advantage of genomic resources to be able to address research questions using existing deposited data rather than using new animals. Our regular lab meetings optimise tissue sharing and banking. Finally, where possible we optimise reagents using in vitro cellular models prior to using them in vivo - for example, when using siRNA or AAV vectors.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project will exclusively use mouse models. Mice are the species of lowest neurophysiological sensitivity that are appropriate for this research, and have well-characterised visual and circadian physiology. Extensive genomic data coupled with a wide range of existing transgenic, mutant and knockout models make mice the only viable model for this work.
Methods primarily involve non-invasive monitoring of physiology and behaviour, which includes home cage activity monitoring using passive-infrared movement sensors, capacitive sensors, infrared cameras, voluntary running wheel activity and thermal imaging. In addition, we routinely use simple non-invasive assessments of vision, including head tracking to visual stimuli, pupillometry and novel object recognition. Some tests - such as electroretinography or retinal imaging - may involve brief reversible anaesthesia. Where magnetic fields are used, these will be generated using a Helmholtz coil around the home cage. A smaller number of studies may involve delivery of substances to the eye or brain, such as AAV vectors or siRNA, to modify visual or circadian responses. These studies will always use the most refined method of delivery and the smallest devices possible, as well as appropriate anaesthesia and analgesia. At the end of experiments, tissues are collected for ex vivo analysis.

Why can’t you use animals that are less sentient?

In mammals, the photoreceptors regulating circadian physiology are confined to the eye. In nonmammalian vertebrates, photoreceptors may also be found in the pineal organ and deep brain, as well as other tissues throughout the body. As such, to be relevant to human physiology, the study of mammals is essential. As the mammalian visual system continues to develop after birth, using earlier life stages is not possible. A key output of our research are empirical measurements of behaviour, which precludes the use of terminal anaesthesia. However, we do routinely study ex vivo tissues, minimising the need for live animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

A key feature of our work is to minimise disturbance to animals, so constantly seek refinements in physiological and behavioural monitoring. To this end, we have already developed and validated many non-invasive methods of studying vision and circadian physiology, including simultaneous activity and sleep monitoring and thermal imaging to study body temperature. These monitoring tools also provide ideal welfare indicators, enabling us to

accurately monitor disturbances of activity, sleep and body temperature throughout our studies. We also have extensive experience in behavioural procedures, and the use of habituation and counterbalancing enables us to minimise any effects of treatment order. We have worked closely with the vets and other collaborators across the Establishment to optimise in vivo substance delivery. For example, for intraocular substance delivery, we use an ophthalmic microscope to enable us to see inside the eye during injections and have optimised our setup to allow inhalational anaesthesia for faster recovery. We routinely use pre-, peri- and post-operative analgesia for surgical procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We have recently published guidelines for the effects of light on mouse physiology and behaviour. We also routinely use published standards for experimental design, such as the ARRIVE guidelines. We also have a track record in data deposition and sharing to allow others to benefit from our data and improve reproducibility.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

As chair of the Establishment's 3Rs committee, I am well aware of developments in the 3Rs across the University as well as nationally and internationally. This role involves working closely with the NC3Rs as well as many other welfare researchers. My lab has played a key role in the development of a wide range of 3Rs advances, including many non-invasive approaches to studying physiology and behaviour as well as methods of using existing genomic resources to address new scientific questions. For example, we have developed non-invasive methods for measuring sleep based upon home cage movement and have recently validated the use of thermal imaging to measure body temperature in mice. We have also pioneered methods of combining existing gene expression data in the form of meta-analyses.

Home Office

NON-TECHNICAL SUMMARY

116. Memory in the rat

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

No answer provided

Animal types	Life stages
Rats	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand the capability of rodent memory, and in particular the role of context in mediating such memory.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

With an increasingly old population, problems with memory are becoming increasingly common. We know different types of memory rely on different structures and systems in the brain, but we still have a relatively poor understanding of what system underlies what type of memory and (as importantly) how it supports that memory. To understand this we need to understand whole systems where different memories can be learned, stored and retrieved in order to be able to help those with memory problems.

What outputs do you think you will see at the end of this project?

The lab has a strong track record of publishing high profile outputs (journal articles, reviews etc) from such work. The aim of this project (to understand the capability of rodent memory) is of broad and timely interest at the moment and therefore a strong track record of publications is expected to come out of this project.

For example, context plays a key part in the control of clinically relevant forms of memory. Episodic memory (for example) can be thought of as the memory for what happened, where it happened and which occasion it happened on, and the contextual factors which separate one occasion from another appear to be critical in sparing memories from confusion and interference. Therefore understanding the nature of context in rodent memory is critically important to understand its relevance to clinically relevant forms of human memory.

As a result, outputs will include new publications (relevant to a wide audience of researchers in different fields), information to improve research methods and reliability and behavioural tasks which may improve the reliability and translatability of research with significant clinical focus.

Who or what will benefit from these outputs, and how?

In the short and medium term the project will deliver high profile journal publications for the academic community. We have a track record of highly cited work in this area and the work in the current project is likely to continue to deliver outputs of broad interest to a wide community of researchers (including those interested in human memory as well as animal memory).

In the short and medium term we would continue to work with commercial partners to adapt our novel tasks into commercially available tasks (if appropriate). This apparatus has significant 3Rs potential, and increasing the market through novel tasks has the potential for further reduction in animal use in the medium and long term.

In the long term objective 2 will deliver clinically relevant tasks of memory in rodents, allowing improved translation of work in animals to the clinic.

How will you look to maximise the outputs of this work?

As well as typical academic publishing routes, the lab has a track record of dissemination to different populations (clinicians, pharmaceutical industry, behavioural testing industry) and the same would be true of the current project. The work will be discussed at a wide variety of meetings, attracting different types of audience. In addition the work will be communicated with existing industrial partners to ensure the maximal impact of the knowledge gained.

Species and numbers of animals expected to be used

- Rats: 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are interested in how different systems in the brain work to give rise to different types of memory. To do this we need to use whole animals - other systems can show that memory is present but only measuring the behaviour of animals shows what type of memory is affected. Therefore we need to use animals that have a brain structure similar to humans, but also that can perform tasks which are relevant to the problems that humans face in the clinic when they present with memory difficulties. For that we use mice and rats, where the brain structure is well understood and has a high degree of similarity with humans and where tasks have been specifically developed to show comparability of their behaviour with human memory. To fully understand how different brain systems support memory we also need to understand how these systems change over the lifespan of an animal. We need, therefore, to look at how these different types of memory and change as animals grow up and grow old, to make comparisons to the problems humans face with memory as we grow older.

Typically, what will be done to an animal used in your project?

Most animals on this project will be tested on behavioural tasks of memory, and to encourage them to work on these tasks they may on occasion have a slightly reduced diet to motivate them to eat food rewards they can achieve in the tasks themselves. Some animals will do these behavioural tasks after they have had surgery to remove a system in the brain related to memory. This allows us to see what the role of these systems is in normal memory performance. These animals will have surgery under a deep anaesthetic and would only have one surgery in their lifetime. No juvenile or aged animals would have surgery, though some animals that have had surgery may be kept for study as they age.

What are the expected impacts and/or adverse effects for the animals during your project?

Behavioural studies are unlikely to cause any ill effect to the animal. Reduced diets to motivate the animals on some tasks cause problems only very rarely, and when weight drops below specified amounts. Animals will be weighed very regularly and so such changes will be spotted before they become problematic and irreversible.

Animals' time in any behavioural test apparatus will be limited (typically 2 hours and no longer than 3 hours) and for the number of tasks learned will be relatively low (e.g. typically around 3-4 tasks over approx. 6 months, although there will be fewer tasks over a longer period where learning is difficult).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All animals run only on behavioural experiments (with or without reduced food diets) are likely only to experience mildly adverse effects.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

There are many different types of memory, and different systems in the brain are likely to support each different type. Therefore, demonstrating the presence or absence of memory (such as whether changes happen between single cells) cannot tell us about the type of memory being affected. To understand that we need to observe behaviour in whole animals where different types of memory can be detected through different tasks.

Which non-animal alternatives did you consider for use in this project?

Because of the need to observe behaviour only whole animal systems can be used, though this can include humans.

Why were they not suitable?

The need to link the behavioural changes to specific brain systems requires surgery to remove those systems before observing the behaviour. As a result such studies would not be possible in humans.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For most studies (whether behaviour alone or using lesions) to understand differences in memory we need to compare one group with another where each group either performs a different task or has a different type of surgery. The numbers here then are arrived at through determining the number of groups that would be run and the number of animals in each group needed to provide reliable and reproducible information in the behavioural tasks (i.e. too few animals wouldn't be enough to demonstrate differences between groups, whilst too many may show effects that are so small as to not be reliable).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will always run an animal on many different tests where possible. This means that we do not need to use different animals for every test (reducing numbers) but it is also a more powerful experimental design which allows us to be more confident in the data we get. Design assistants such as NC3R's experimental design assistant can be helpful in informing the best design to minimise numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We are also very experienced in using tasks and lesion methods that reduce the number of animals needed, by making tasks collect more data more reliably and by reducing factors that can impact on the tasks other than the measure we are trying to observe.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Behavioural testing is unlikely to lead to significant pain or distress. Our surgical techniques are refined to minimise the risk of post-operative effects.

Why can't you use animals that are less sentient?

We need animals that can complete tasks which are relevant to humans. Without this we face a problem where what we learn from animals may not be relevant to problems in humans. Rodents provide us with a level of behaviour which we have evidence relates to human performance.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Post-operative care and pain management are carefully monitored throughout and surgical procedures allow us to minimise these (for example by giving pain relief before as well as after surgery). We also have experience in refining the behavioural tasks to minimise handling and the stress associated with it.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The project will ensure that ARRIVE guidelines are followed at all times. The lab is well aware of these guidelines and follows them in its current practice. They will be continued to be followed and all researchers working on the project will be committed to understanding these guidelines and the relevance of them to experimental design and running.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The lab has developed several projects funded by NC3Rs and is closely linked to current advances in 3Rs as they apply to this work. All people working on the project will continue to develop their skills and working methods through the sharing of best practice within the lab and the wider community.

Home Office

NON-TECHNICAL SUMMARY

117. Metabolic and Endocrine Pharmacology

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Metabolic, Endocrine, Diabetes, Pharmacology

Animal types	Life stages
Rats	adult
Mice	adult
Guinea pigs	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To evaluate the effects of both pharmaceuticals and other substances (e.g. Industrial Chemicals) on Metabolic and Endocrine function. This may be both for efficacy and safety purposes.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In the case of efficacy, there are numerous conditions in humans that are caused by diseases or dysfunction of metabolic and endocrine systems (e.g. obesity, diabetes, high cholesterol) with either an unmet clinical need or drugs that are not wholly effective and have undesirable side effects. This project will seek to help in the development of better, safer therapies with less side effects.

In terms of safety, industrial chemicals (for example) have been shown to affect both male and female reproduction, and evaluating their safety is required by law, to ensure the safety of the public from their effects. It is also possible that new drugs for other indications may have undesirable effects on the metabolic and endocrine systems, and we may undertake testing of such drugs, to determine their safety in this programme of work.

What outputs do you think you will see at the end of this project?

The benefits of this programme will be two fold. Firstly it will contribute to the development of new drugs that help alleviate metabolic and endocrine disorders (such as diabetes and obesity). These new drugs may work better in the clinic, relieve or cure diseases and have better side effect profiles. We may, by our work, also contribute to better knowledge and understanding of these types of disease, and that knowledge may be used to develop further new drugs.

Secondly, this programme also allows for the assessment of various chemical classes regarding their ability to produce endocrine disruption (e.g. industrial chemicals, agrochemicals and industrial solvents.) Found in many household and industrial products, endocrine disruptors are substances that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for development, behaviour, fertility, and maintenance of homeostasis (normal cell metabolism). These hormones include both oestrogen and testosterone. In recent years global regulators (who oversee the quality and safety of chemicals and pharmaceuticals) have increased their interest in such chemicals and have issued directions for the testing of already marketed chemicals . The scope has also been widened to include certain classes of chemical e.g. pharmaceuticals.

On some occasions, where previous studies have shown that a compound may affect metabolic or endocrine function, the models on this project maybe used to assess the safety of a test material and find a dose that causes no effect. This is important when planning future trials in humans, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Who or what will benefit from these outputs, and how?

In both the medium and long term, patients will benefit by having newer, modern and safe drugs that are more effective than the current options. Humans will also be safer knowing that certain chemicals have been risk assessed and classified as safe or not, as potential disruptors of their endocrine function.

How will you look to maximise the outputs of this work?

The work will be shared with customers who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used for regulatory purposes or to support regulatory purposes (e.g. to support drugs progressing to clinical trials, or to show that a certain chemical is safe for human exposure). Previously however, we have collaborated with customers and shared data we have produced in the form of Scientific publications that are in the public domain.

Species and numbers of animals expected to be used

- Rats: 7500
- Mice: 1500
- Guinea pigs: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Most of our experiments will be carried out on mice and rats as these are the smallest relevant species that we can use that have a metabolic and endocrine system that is comparable to humans. In some specialist cases we may use other animals (the guinea pig) because what we are trying to find out is better done in that particular species rather than in the rat or mouse.
The only other time we would use a species other than a mouse or rat is to continue work that has been previously done in that species. For instance if previous work, and results gained, had been carried out in a guinea pig, it would make no scientific sense to start the next stage of a programme of work in a rat or a mouse.

In some protocols we may use genetically altered animals to investigate some types of metabolic disease like diabetes and obesity. This because these alterations mean the animals produce symptoms and characteristics of these diseases that we see in humans like higher than normal levels of blood sugar in diabetic mice. The use of genetically modified animals is rare.
We will be using adult animals in our studies, as we do not expect to be investigating either metabolic or endocrine disease in young animals or children.

Typically, what will be done to an animal used in your project?

Typically on this project, animals are dosed over a period of time with test materials, and sampled (e.g. blood or urine) before having tissues taken after they have been humanely killed. Most studies would last a matter of days (much less than a month) although some, occasionally may last for longer than that.
Dosing of animals is commonly done orally using a flexible tube, or by injection using a syringe and needle, maybe directly into a vein, or into a muscle into the arm or leg, or just under the skin.
Blood samples are usually taken from easily accessible veins in the neck or the tail of rats or mice. We are limited to how much blood we can take at once or over a month. If we need a large blood sample, we would do this when the animal is anaesthetised and we would not let them recover consciousness.
Where possible, we try and take as many of the tissues and samples we need after the animals have been humanely killed.
Some animals we used are genetically altered, so they better represent disease states like diabetes in humans.

Sometimes we need to induce a metabolic condition in animals to test if a new drug can alleviate the condition. This can be done with diet or maybe by injection. When this happens we monitor the animals closely to check they don't get ill.
In some protocols we also have to surgically prepare animals for testing by removing their ovaries or testes. These tests are specialist to check for the potential adverse effects of chemicals and other substances on male and female sex hormones, and are required by law. To measure glucose levels in rats, sometimes we surgically implant an electronic device which means we can monitor blood glucose levels continuously without having to take many blood samples.

What are the expected impacts and/or adverse effects for the animals during your project?

When dosing an animal by injection or taking blood, The amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.
Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a vet before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.
The genetically modified animals we use are usually models of type II diabetes. These animals are heavier than normal, urinate more frequently, and are more sedentary than normal animals. They don't look or behave like they are ill however.
Very occasionally we may need to take a urine sample for analysis, so we would then put an animal into a special cage which is smaller than their normal cage. The animal can still move around however, and we'd normally introduce an animal to this cage to acclimatise them to it. Virtually every animal will get used to their new cage within about 15 minutes and are fine.
Dosing with drugs and chemicals may cause adverse effects in some studies, although this is rare. We do observe our animals at least twice a day, and the people who do this know the signs when an animal is ill. If an animal is ill, we would check it more frequently, and get more senior staff involved in its care for advice, including vets. We also help sick animals out by giving more bedding, more heat and special food to make them more comfortable.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

On the last project, about 80% of animals were classified as having displayed moderate severity. This is because legally, all surgical procedures carried out on an animal must be classified this way. the rest of the animals were classified as having displayed mild severity.
It's impossible to predict the proportion of severities expected on a service licence, as this will be dependent on what study types we are asked to perform.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The endocrine system and other systems influencing metabolism are complicated and perform a wide variety of functions essential to life, like maintaining blood glucose levels, or keeping hormone levels correct for normal daily life. There are no adequate models to replace the whole animal experimental model, as the complexity of the human body cannot be fully replicated in a test tube.

In many cases the protocols listed in this Project will be used later in the life cycle of a test substance and in many cases, particularly for pharmaceuticals, tests in test tubes will have been conducted previously (often by the Sponsors) to ensure the drugs do what they are meant to, and are safe, before we expose them to testing in animals.
For some protocols the licence, the tests are legally required by regulators around the World to make sure certain chemicals and other substances don't have ill effects on male and female sex hormones, if you are exposed to them by accident or otherwise.

Which non-animal alternatives did you consider for use in this project?

There are no other non-animal alternatives for the work being undertaken on this project.
For some protocols, there are tests carried out in test tubes that determine whether or not chemicals and other substances may pose a hazard to male and female sex hormones. It's only when this hazard has been identified, that the next step in the testing tiered process (in animals) is triggered.

Why were they not suitable?

Although there are test tube tests that can model some parts of how drugs or chemicals get into our bodies, and how our body deals with them, for example, there is no one test tube test that brings all these complex happenings together, like we see in animals and humans.
That's why we need to test some substances in animals, as they have similar physiology and endocrine/metabolic processes as humans, and that testing gives us a good idea what may happen if they were ever tested in, or exposed to humans.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from customers in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature will normally be consulted to help establish the group size. Alternatively, there may be other data to aid this process. The Department of Statistics and Data Management are often consulted to assist in this process particularly where the study type is not routine.

Whenever possible, common control groups will be used in order to minimise the numbers of groups used.

For less established study types, preliminary pilot studies may be conducted whereby smaller numbers of animals may be used to generate data in order to ensure that the experiment operates to expectations and to generate some data which may be used to optimise the study design.

Experience has shown that occasionally, Sponsors have a preference with regard to their design and numbers of animals to be used. Rationale for the design will be requested from the Sponsor and such designs (particularly where they are at variance with EU requirement or studies usually conducted here) will be discussed internally (and the Home Office as appropriate) and forwarded to the Department of Statistics for advice. Such advice will be taken into account when determining the design/numbers to be used in the study with the goal of using the least number of animals to achieve the scientific objective.
In addition, the regulatory test guidelines used for testing in some protocols, stipulate group sizes (a minimum of 6). These are safety tests to evaluate whether chemicals and other substances have any effect on male and female sex hormones.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For studies where a new drug is being tested in animals for the first time, we would often test that in a small group of animals (usually 3-5) to give us confidence that the dose levels we chose are safe, and the drug affects the system its designed to, without making an animal ill. These are called pilot studies.
We will try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to get a blood glucose level in the blood , or if we need to find blood borne markers of diabetes, for example, we will often do that in the same animal, rather than use separate ones, when possible.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Most of our models involve dosing animals with test materials, and sampling them, with many outputs taken after the animals have been humanely killed. This is generally the least invasive set of procedures that can be done to give meaningful outputs to make scientific decisions about further tests.
In some tests we use animals that are genetically altered, to mimic conditions seen in humans we are investigating like diabetes and obesity. Sometimes we alter the existing conditions of animals by introducing a

drug, or changing their diet. This doesn't cause harm on its own, but the consequences of these may cause a mild degree of suffering to the animals.
We also use some surgical models to investigate the effects of chemicals and other substances on male and female sex hormones. For male animals this surgery (castration) or female animals (removal of the ovaries) are both the best ways to determine if a test material has an effect on male or female sex hormones, even though surgery is involved. The use of non surgical models (using immature animals who don't have fully developed testes or ovaries) was also considered, but these models are not always accepted by regulatory authorities for reasons of female endocrinology , and their timelines for dosing are so exacting (specific dosing dates after birth must be used), than to use them would mean us having to use more animals than if we used surgical models.

Why can’t you use animals that are less sentient?

Rodents (rats, mice and guinea-pigs) will be used in all of the studies conducted under this licence. Rodents are considered to be of the lowest neurophysiological sensitivity that will allow us to achieve the study aims and are considered suitable for the predicting what's likely to happen in humans

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study.
In addition, care is taken to provide as much environmental enrichment as possible . This is things like plastic shelters in their cages, blocks to gnaw on, extra bedding for warmth and if they need it, food supplements after surgery.
With glucose measurements as over the counter glucose monitoring devices have increased in accuracy, it has been justified, in most cases, to switch to the use of such devices when measuring glucose level. These are the devices that humans use to measure their own blood sugar, given to them by a doctor. This has allowed the volume required for a glucose measurement to be drastically reduced from approximately 0.3 ml to as little as 0.01 ml. In addition to the obvious benefit to the animal whereby far less blood is required for an individual sample. It also means that we can use the same animals to get a time-course of action of any drug, instead of having to use several groups. We can sample on multiple occasions by removal of the scab from the initial wound on the tail and analysis of the small drop of blood that results which is sufficient for the analysis thereby not necessitating further needle stick penetration into the blood vessel.
  The use of a surgically implanted telemetry transmitter in rats, means that blood glucose levels can be tracked electronically, and that no blood sampling is required during the course of a study.
Dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm and will be the minimum consistent with the scientific objectives. In addition, suffering will be further minimised by implementing clearly defined humane endpoints
.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

For any surgical interventions, then the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) will be followed.

For blood sampling and dosing then the following guidelines/literature will be followed:

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23 (2001).

Regulatory guidelines

OECD 440 Uterotrophic Bioassay in Rodents:
A short-term screening test for oestrogenic properties.
EPA OTTPS 890.1600 'Uterotrophic Assay'.

OECD 441 Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties.
EPA OCSPP Guideline 890.1400 Hershberger
Assay.
ICH Topic M 3 (R2)
. Non-Clinical Safety Studies for the Conduct of
Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

Home Office

NON-TECHNICAL SUMMARY

118. Mice with adaptive human immune systems and their application for drug and vaccine discovery

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Antibody drug, Vaccine, Cancer, Covid 19, T cell

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this project is to genetically alter mice to humanise their immune system and subsequently use such mice to identify and develop novel drugs for treating diseases and vaccines for disease prevention in humans.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to undertake this work so that we can discover new drugs and better vaccines for treating and preventing many different diseases in people.
Mice with human immune systems respond to vaccination just like people do. Therefore, by testing different vaccines in mice with human immune systems we can identify better vaccines that are much more likely to work in people.
We can also isolate human antibodies from these mice. These can be developed as drugs to treat diseases like those caused by coronaviruses such as Covid 19 or cancers like leukaemia and prostate cancer.

What outputs do you think you will see at the end of this project?

New medicines, namely vaccines and drugs.

- Drugs that offer potential new treatments for a form of leukaemia.
  Vaccine candidates that offer improved protection against malaria
- A second generation Covid 19 vaccine, improving on the proportion of vaccinated people who become immune to the virus following vaccination and improving on how long this lasts.
- **Basic Science benefits**:

- Publications in the peer reviewed scientific literature
- Genetically engineered mice that can be widely used by the scientific community to pursue related projects in other disease areas.

- Grants to support continuity of the work for an additional funding period.

Who or what will benefit from these outputs, and how?

- All countries of the world could benefit from the discovery of new information that supports the development of vaccines for Covid 19.

- Societies in countries where malaria is endemic could derive benefit (in the long term) if we are successful in identifying a better vaccine for malaria, recognizing that it can take many years for a result in the laboratory to be shown to provide long term benefit in humans.

- Society will benefit from knowledge generated as this provides better understanding of diseases and methods to treat and/or prevent them.

- Research institutes, pharmaceutical companies and non-governmental organisations will be able to take information produced by such studies and use it to support the development of drugs or vaccines that ultimately provide benefit to patients.

How will you look to maximise the outputs of this work?

We will collaborate with experts in the field, for instance our work on vaccines is a collaborative endeavour with a variety of laboratories.
We will publish scientific papers, thereby disseminating knowledge.
We will file patents, thereby placing in the public domain detailed knowledge of the discoveries we have made.
We will raise money so that the development of promising medicines can continue.
We will out-license promising drugs in situations where we cannot afford to develop these drugs ourselves, thereby allowing the benefits to emerge.

Species and numbers of animals expected to be used

- Mice: 38,550

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse was selected for this project because of the availability of advanced technologies to make "transgenic" mice. Transgenic mice are mice in which their genome has been genetically altered. This is accomplished by adding or removing genetic information at very early embryonic stages. The methods used to do this are very efficient and extensive changes can be made. The refinement of these techniques mean that many fewer animals are needed compared to achieving the same goals in another species.
The immune system of the mouse is similar to that of humans so we can replace mouse genes with the equivalent human ones to make mice that have a more "human relevant" immune system. Although very large stretches of mouse DNA has been replaced with the human equivalent the human genes substitute for the mouse ones. The resulting mice do not exhibit any signs of suffering or distress. The other genetic changes we will make in this project involve removing genes for drug targets from the genome, which should not adversely affect the mice either. Adult mice are used for vaccinations because their immune systems are mature.

Typically, what will be done to an animal used in your project?

To produce transgenic mice fertilised embryos are collected which are manipulated under the microscope. These are transferred to foster mothers who become pregnant, give birth and rear their pups.
The embryo transfer is done under general anaesthesia as we need to make a small cut in the skin to place the embryos in the uterus or oviducts. Typically, the mice recover very quickly from surgery, they are actively running around, eating and drinking after 1 hour and are fully recovered within a few days.
A small number of male mice are vasectomised, which are used to mate with foster females.
Vasectomy is conducted on anaesthetised males by making a small scrotal incision and cutting the thin tube carrying the sperm. The incision is closed and the mice recover from the anaesthetic within 60 minutes or so. A week or two later the wound will be healed and the mice can be used for mating with females.
Transgenic mice in this project will be bred and weaned. Where the line is not pure bred a small piece of tissue from the ear is taken for genotyping the mice to identify the genetic alterations they carry.
Transgenic mice are used for vaccinations also known as immunisations. Vaccination involves injecting a purified foreign substance into the mice, which is known as an "antigen". The vaccinated mice develop antibodies against the antigen and a types of cell known as a T cell begin to divide too.
Immunisation is usually conducted with a very fine needle and a small volume as this is the least painful method. To get a good immune response usually we conduct several vaccinations over a few months, similar to the "MMR" vaccinations we give our children. To check if the vaccination is working a small drop of blood is collected during the immunisation which we check in the laboratory to see if we can find antibodies that recognize the target. In the final stage, the mice are killed and the blood, spleen, bone marrow and lymph nodes collected for analysis.
In some cases instead of purifying a foreign substance from a virus or bacteria for immunisation, it is more efficient to take the gene and put this in the mouse. This is like gene-therapy in that we inject DNA encoding the foreign substance into a blood vessel which then ends up in the liver where it expresses the foreign substance for several weeks, resulting in a strong immune response. This is similar to the famous Covid 19 vaccine trial from Oxford, in which the DNA that makes a Covid 19 protein is used to vaccinate human subjects.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the animals in this project should not experience any adverse impact as they are used for mild procedures like breeding and vaccination.
The procedures used for immunisation involve needle pricks for vaccination and to collect small amounts of blood for analysis. Other procedures used for immunisation may be involve injection into a vessel which may involve anaesthesia. In either situation the mice are subdued and lethargic for an hour or two but then recover fully as the affects of the injection and/or anaesthesia wear off.
Some of the animals will experience transient pain from the minor surgery required for embryo transfer and vasectomy. Both of these procedures which will be conducted under anaesthesia and managed with drugs to relieve pain.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mouse : Mild severity : Greater than 98%
Mouse : Moderate severity: less than 2%

What will happen to animals at the end of this project?

- Used in other projects
- Killed
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The immune system is extremely complex. We have two main types of cells that recognize and kill bacteria and virus infected cells known as B cells and T cells.
B cells make trillions of different antibodies which are released and circulate in the blood. When they find an infected cell they attract other cells to the site of infection to begin an immune response to kill the virus or bacteria. Each B cell makes a different antibody, but if it encounters a foreign substance on the surface of a virus or bacteria (known as a foreign-antigen) and it is able to bind to it, the antibody produced evolves so that its binds more and more tightly. By the end of an infection the antibodies our B cells produce bind very tightly to their target and are therefore much more effective at killing it.
T cells roam around the body looking for infected cells which they recognise through a unique structure on their surface known as the T cell receptor - which has some similarities to an antibody. Like B cells, every T cell has a different receptor and we have trillions of different T cells receptors too. T cell receptors recognize and bind to foreign antigens, but only do after these have been chopped up into much smaller pieces and presented on the surface of the infected cell. When a T cell encounters an infected cell it starts to divide, it releases chemicals to attract many other types immune cells - such as B cells - and they can also directly kill the infected cell.
The immune response is highly complex. It matures over many months. It involves the interaction of many different cell types and migration of cells to a variety of sites in the body. The interplay between the different cell types simply can't be emulated in the laboratory. This project uses immunisation with foreign antigens to explore the maturation of the immune response, it is not possible to use experimental vaccines in humans.

Which non-animal alternatives did you consider for use in this project?

Some artificial methods using bacteria can be used to discover antibodies in the laboratory, the most well-known of which is a technology known as phage display. In this technology viruses that infect bacteria are modified to carry antibody fragments. Large collections of these, known as "phage libraries “, can be generated using the genes from human antibody producing cells. Similar technologies have been developed using yeast.
Phage and yeast libraries can be examined to find individual members which bind to a drug target but the antibodies in these libraries are **immature**. They don't bind to their target strongly and they can lack specificity because they bind to other targets, which would cause side-effects.
Antibodies isolated from immunised humanised mice are different - they are **mature**. The difference comes about because the mouse derived antibodies have been induced to evolve into a mature form by immunization. **Mature** antibodies make much better drugs because they bind much more tightly (1000- times or more) to their target - such antibodies can be developed as drugs without further modification.
Artificial methods can also be used to make libraries of T cell receptors from human T cells. However, these libraries will not have T cell receptors that bind to human targets as these are normally removed from the body when a T cell matures otherwise we would all suffer from autoimmunity. However, human T cell receptor genes that bind to human targets can be found in mice with human T cell receptors.
Although these artificial methods provide some options, they can only be experimentally used in isolation. As summarised (and simplified) above, the immune system matures a response, selects and matures ideal antibodies and unique T cells though a highly complex series of interactions involving many different cell types that naturally evolve in an animal over many months. Simply, there are nonanimal alternatives that can adequately emulate this highly complex process.

Why were they not suitable?

Artificial display systems can be used to discover antibodies, but in practical applications, these methods have several severe limitations which restricts their usefulness. They are:

- They can bind to several targets, rather than just one.
  They bind weakly to their targets.
- It is difficult to make large quantities and concentrate them.
  It's very hard to find antibodies or T cell receptors which bind human targets.
- These issues have to be resolved in the laboratory. This is a slow, error prone, unpredictable and often fruitless process. As a result, despite huge effort only 5% of almost 100 approved antibodies for human
- clinical use have been derived from phage libraries.

Similar issues have been encountered using phage-based methods to turn human T cell receptors (TCRs) into drugs. Powerful and uncontrollable off-target toxicities and human fatalities have resulted.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals estimated to be used for this project are based on 35 years of experience in generating mice with altered genes. They are also consistent with the usage in the existing project license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

More than 90% of the animals used in this project will be used for establishing and breeding complex combinations genetic alterations.

- Where possible we establish mice which breed true. In these mice both copies of each genetic alteration will be the same.

- As our genetic alterations are made step-wise, we try to completely finishing one before crossing them together.
- We test the function of each genetic alteration before breeding them together.
  The construction of large humanized alleles requires many cycles of genetic engineering. We use
- embryonic stem (ES) cells for this purpose. We constantly select for ES cell clones that perform well, thereby reducing mice used for donor embryos and recipients.

The other use of mice in this project is for the immunisations required to deliver the core scientific objectives. To reduce mouse numbers we:

- Use standard operating procedures for immunisations and tissue harvesting, ensuring that we get the maximum amount of useful biological materials from each mouse.

- We consult widely to identify and where appropriate integrate improvements in methods to maximise the recovery of data from every mouse

- We have invested in technologies which enables us the sequence the antibody and T cell receptor genes in single cells. By using this technology we get much more information from every immunized mouse. Some years ago we would recover a handful of useful antibodies from each mouse. Today we are able to recover 100-200, which means the mouse numbers required are much lower than they were previously.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

There are four measures we take to reduce mouse numbers:

- We use colony management software to keep track of mice in the animal room, set up just the right number of breeding pairs to produce the mice we need. The data base will record pedigrees and procedures - keeping an electronic health record.

- We conduct pilot immunisations with antigens we have not used before to assess their performance before initiating larger experiments or not conducting them at all in cases where the pilot does not provide a good basis for continuing.
- We stop breeding and preserve mouse lines in cryogenic storage if they are no longer required

- We use contemporary methods to make genetic mutants which avoids numerous cycles of mouse breeding.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice with human antigen receptor loci

The genetic alterations that we have introduced into our mice are mostly mouse/human gene replacements. The mouse genes are exchanged for the human ones which are very similar. Although the resultant mice have a human immune system it is fully functional and consequently the mice are totally healthy. They do not experience any immunological deficiencies.
We also genetically remove the genes for drug targets from the genomes of these humanized mice. This improves the immune response against the drug target. To minimize any unexpected consequence of this type of genetic modification, we carefully check the literature and databases and will not proceed if the mice are not expected to develop normally.

Immunisation Methods

The immunisation methods we use are highly refined and are widely used. We use standard operating procedures and state-of-the-art downstream methods to get the most out of every immunized mouse.

A significant amount of work is performed in the laboratory to maximize the chance that an antigen is able to elicit a good and meaningful immune response. This includes ensuring that the antigen is pure, free of toxins and has not been damaged during its preparation - for example it is has not been degraded or aggregated together. A significant amount of animal use from repeat immunisations is avoided by rigorous application of these principles.

Similarly, carefully preparing the antigen so that it is free of toxic or inflammatory agents avoids unnecessary suffering and focuses the mouse's immune system on the relevant target.

Why can't you use animals that are less sentient?

The immune systems requires several months to develop a robust immune response against a target. The immune system is not mature at an earlier stage (immature life stage).
Lower organisms can't be genetically engineered to the extent possible with mice, the mechanisms of an immune response are different and the structure of the antigen receptors are also different.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I don't anticipate many refinements to transgenic methods as these are already very standardised and efficient, though where these are reported we will test and implement them.
Where possible we will employ highly experienced animal care staff who are familiar with the specific strains, experimental activities and careful handling of the mice. Familiarity with the balance between welfare and experimental needs, observing recording and reporting expected and unexpected outcomes at the cage-side with score sheets is another essential aspect of minimizing welfare costs. An important balance needs to be struck between to much disturbance of the mice while allowing time pre- and post-procedures for acclimatisation and recovery.
Where possible the mice will not be disturbed unless required for routine husbandry, daily checks or experimental purposes. Environmental enrichments like tubes and nests will be provided.
Where possible mice will be acclimatised, for instance when moved between facilities or animal rooms before any experimental procedures are conducted.
We consult existing literature to ensure we use the latest refinements in experiments. Work will be carried out in state-of-the art facilities by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare.
The scientists and technicians work closely with the trained and highly experienced personnel in the facility and the veterinary surgeon to ensure that animals experience minimal adverse effects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I use guidance from the following sources:

- The National Centre for the Replacement Refinement and Reduction of animals in Research (NC3Rs) https://www.nc3rs.org.uk

- The International Mouse Phenotyping Consortium (IMPC) https://www.mousephenotype.org.
- The International Society of Transgenic Technologies (ISTT).
- Some of my team members are members of The Laboratory Animal Science Association (LASA) and attend an annual conference where information on best practice is often exchanged

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I periodically check website for The National Centre for the Replacement Refinement and Reduction of animals in Research (NC3Rs) https://www.nc3rs.org.uk which has many excellent standard operating procedures and videos.
I'm also a member of an international society where there is frequent technical "chatter" regarding technical advances in the field observations and experience implementing these.
Implementation of a technical variation will usually be conducted with a pilot experiment to gain confidence in the actual method and its reported advantages, ideally with suitable controls. Once this has been assessed and shown to be an improvement then this will be introduced in the standard operating procedure and then implemented as a routine.

NON-TECHNICAL SUMMARY

119. Mice with human antibody genes: Drug discovery and vaccine assessment

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Therapeutic human antibodies, Vaccines, Infectious diseases, Cancer, immunology

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Therapeutic human antibodies will be isolated and vaccines tested using mice with human antibody genes.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Antibodies have been used as medicines for more than 125 years. For most of this time antibodies from immunized horses and cattle has been used. This has provided effective treatment for some indications and is still used today as anti-venom for snake bites.
With the advent of modern molecular methods it has been possible to isolate individual antibodies and develop them as drugs for a wide range of diseases. Isolated antibodies can have a very high specificity and are very stable drugs, requiring dosing just once per month. The problem, however, is that if one needs to administer the antibody more than once, it can't come from an animal source as its foreign nature will be recognized by the human immune system and it will be strongly rejected.
To overcome this problem it is necessary to use a human antibody. Several methods have been used to make human antibodies. In some cases bits and pieces of an antibody from a mouse or rat antibody have been stuck together with parts of a human antibody to make them appear human. In some cases this is effective but its not easy to make a mouse antibody look like a human one. One of the best sources of human antibody drugs are mice in which the mouse genes which encode for antibodies have been replaced with ones from humans. Some mouse strains have very complete coverage of the large human antibody genes.
We use these mice, which can make trillions of different human antibodies, to discover antibodies which we can develop as drugs for a variety of serious diseases like cancer, autoimmunity and infectious diseases.
Mice with human antibody genes, have immune response that mimic those of a human. This is useful for testing experimental vaccines to make sure they work before they are used in humans.

What outputs do you think you will see at the end of this project?

The main output of this project will be new medicines, namely therapeutic human antibodies and vaccines.
The discovery, development, clinical testing and approval of therapeutic antibodies and vaccines typically takes ten years or more. Thus, at the end of the project, antibodies for treating several diseases and protective vaccines will have been identified, tested in preclinical models and some will be in advanced stages of clinical testing. For many of these antibodies and vaccines we anticipate publishing pre-clinical and clinical results in the peer reviewed scientific literature.
Antibodies emerging from our work on SARS-Cov-2 and the vaccines that we are currently evaluating will likely progress much more rapidly through clinical trials and hopefully will be approved on an accelerated time scale.

Who or what will benefit from these outputs, and how?

The long-term benefits are drugs and vaccines which will directly benefit human health**.** These benefits will play out over a longer time scale than the temporal envelope of this project. Human therapeutics typically take 10 or more years from concept to approval. Vaccines can take longer.
The scientific community will benefit from the new knowledge that emerges from the pre-clinical and clinical studies around each of the projects.

How will you look to maximise the outputs of this work?

We will collaborate with experts in the field, for instance our work on vaccines is a collaborative endeavour with a variety of laboratories.
We will publish scientific papers, thereby disseminating knowledge.
We will file patents, thereby placing in the public domain detailed knowledge of the discoveries we have made.
We will raise money so that the development of promising medicines can continue.

We will out-license promising drugs in situations where we cannot afford to develop these drugs ourselves, thereby allowing the benefits to emerge.

Species and numbers of animals expected to be used

- Mice: 71,200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse was selected for this project because genetic technologies have been developed in the mouse over the last 35 years to an extraordinary degree of sophistication and efficiency. The refinement of the experimental design and techniques for genetically manipulating mice mean that many fewer animals will be needed compared to reaching the same goals in another species.
The immune system of the mouse is similar to that of humans and we know that we are able to replace mouse genes with their human counterparts to make a mouse that has a more human relevant immune system. This is a relatively benign change to the mouse and should not introduce any suffering or distress. The other genetic changes we will make in this project should not adversely affect the mice. Where possible we will search databases to find out how a change in a gene might affect the animal before we do our experiments. If the change is likely to cause problems, we will change our work to try to avoid welfare issues.

Typically, what will be done to an animal used in your project?

To produce transgenic mice females will be mated and 1-3 days later killed and their embryos collected. In some cases, the females will be stimulated with hormones to produce larger numbers of embryos before they are mated. This will require two injections spaced 2 days apart. The embryos that are collected are manipulated under the microscope and then transferred to foster mothers (previously mated to vasectomized males) who become pregnant, give birth and rear their pups until they are old enough to be weaned.
The embryo transfer is done under general anaesthesia as it involves a small incision in the skin and body wall to access the uterus or oviducts. Following the embryo transfer, the incision is closed with sutures and the skin is closed with surgical clips. Typically, the mice recover very quickly, they are actively running around after 1 hour or so and the clips usually fall out spontaneously after a few days, or if not they will be removed within a week.
A small number of male mice are vasectomised, which are used to mate with foster females for embryo transfer. Vasectomy is conducted on anaesthetised males by making a small scrotal incision and cutting the thin tube which connects the testes to the penis. The incision is clipped or sutured and the mice recover from the anaesthetic within 60 minutes or so. The clips usually fall out after a few days if not they are removed. A week or two later the mice are used for mating with females once or twice each week.
Transgenic mice in this project will be bred and weaned. Where the line is not pure bred a small piece of tissue from the ear is taken for genotyping the mice.
Transgenic mice are used for immunisations so that they develop antibodies. This requires a few injections spaced a few weeks or a month apart typically extending over a period of 2-4 months. A small drop of blood will be collected at several time points during an immunisation to monitor the response to the immunization. In the final stage, the mice will be killed and the spleen, bone marrow and lymph nodes collected for analysis.

A small number of immunised mice will be challenged with an infectious virus to test if they are immune or not.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the animals in this project should not experience any adverse impact as they are used for breeding and immunisations. These procedures used for immunisation cause minor transient pain from a needle prick or a small cut to draw blood.
Some of the animals will experience pain from minor incisions, for instance for embryo transfer and vasectomy which will managed with analgesics. The pain in these cases is not expected to be severe or long lasting.
A very small number of mice will be challenged with influenza or another virus. These viruses induce weight loss in mice over a period of several days to one week. Generally, the mice will recover and regain the lost weight during the following week.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority [99%] of mice in this project will experience mild severity procedures.
A minority [less than 1%] may experience a procedure with moderate severity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The immune system is extremely complex. It begins to develop before birth and continues to develop throughout adult life. The components have been shaped by exposure of our ancestors to pathogens over millions of years. The development of antibodies that emerge following exposure to a target antigen over many months of an immune response, is a feature that simply can't be emulated in a laboratory as this involves the interaction of many different cell types and migration of cells to a variety of different sites in the body.

Which non-animal alternatives did you consider for use in this project?

Some artificial methods using bacteria been proposed that can be used to discover antibodies in the laboratory, the most well-known of which is a technology known as phage display. In this technology libraries are generated using molecular biology methods from human antibody producing cells. These libraries can be screened to detect molecules which bind to a target of interest, but the antibodies which can be isolated are immature and can't be directly developed as drugs. In contrast antibodies that have come from humanised mice are mature and they can be developed as drugs without further meddling.

Why were they not suitable?

Theoretically phage and yeast based antibody display systems can be used to discover antibodies, but in practice, these methods have severe limitations. The limitations are not well advertised but there are underlying issues with the technology. The antibodies in phage libraries are immature, which means they lack specificity to their target. Their binding affinities are very poor and their biophysical properties are highly problematic
These limitations are apparent from the very small number of licensed antibody drugs that have emerged from more than three decades of application of this technology by large teams in numerous pharmaceutical companies. Even the best known "phage antibody companies" have been using antibodies from mice. In contrast drugs from synthetic antibody platforms have frequently failed in late stage clinical development.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals estimated to be used for this project are based on 30 years of experience in generating mice with altered genes. They are also consistent with the usage in the existing project license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

More than 70% of the animals used in this project are a consequence of breeding mice with complex combinations of alleles. Where possible we will try and breed homozygous mice with stable allele combinations, but as these strains are evolving it is hard to avoid extensive breeding.
The second most extensive use of mice is for immunisations, which are at the core of the scientific objectives. Many of these mice are used to assess vaccines. To gain statistical significance we need to immunize many mice to ascertain convergence of antibody responses. Investment in technologies to enable deeper mining of immune responses enables greater sensitivity and may in time allow fewer mice to be used per antigen.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

There are three measures we take to reduce mouse numbers:

1. Knockouts are made directly by zygote injection in mouse strains which carry all the other required alleles. This significantly reduces breeding and reduces timelines, although the variability of the method does mean that more alleles need to be carried forward in parallel.

2. We continue to isolate ES cell lines carrying combinations of alleles and use these as a background for targeted modifications thereby reducing allele complexity to n=1 and breeding time to 9 months or less.

3. We cryo-preserve mouse lines which are no longer in use.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs

(harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice with human antibody loci

The mouse was selected for this project because genetic technologies have been developed in the mouse over the last 36 years to an extraordinary degree of sophistication and efficiency. The refinement of the experimental design and techniques for genetically manipulating mice mean that many fewer animals will be needed compared to reaching the same goals in another species.
The genetic alterations that we have introduced into the mice, although rather extensive are benign. The reason for this is the immune system of the mouse is similar to that of humans and we know that we are able to replace these mouse genes with their human counterparts. These mice are fully immune competent; thus they do not experience any immunological deficiency.

Mice with knockouts

In some cases we will make knockouts of target antigens in the genetic background of these humanized mice. This improves the immune response against the target, enabling us to get more and better antibodies from each immunized mouse. It should be noted that the drug targets selected must yield viable knockout mice as healthy mice are required to generate a good immune response. To minimize the impact of this genetic modification, we carefully check the literature and the IMPC database which provides an expectation on the phenotype of the knockout.
It should be noted that although IMPC is incomplete its coverage of the genome continues to expand. In cases where a phenotypic impact is expected, we will design alternative alleles with minimal consequence on the phenotype of the mice.

Immunization Methods

The immunization methods used are highly refined are widely used. Minimization of impact is achieved by using standard operating procedures and highly refined downstream methods to get the most out of every immunized mouse – thereby reducing mouse numbers.
In terms of the antigen, a significant amount of in vitro work is conducted before an immunisation starts to maximize the chance that the antigen is able to elicit an immune response. This includes ensuring that the antigen is properly folded into a native structure and presented along with relevant components – for instance by co-expression of co-receptors, removal of exogenous tags etc. By application of these principles, this ensures that the immune system is directed at an accessible part of the molecule. This may sound obvious, but a significant amount of animal use from repeat immunisations is avoided by rigorous application of these principles. Similarly, by focusing the immune system up front one can avoid going down the long pathway of drug development with a candidate that may eventually fail – with concomitant waste of animals during the testing phase.
Similarly, carefully preparing the antigen so that it is free of toxic or inflammatory agents avoids suffering and reduces animal numbers.

Refined down-stream antibody discovery methods

We have developed highly refined downstream methods for antigen receptor discovery. Some years ago, this would have been achieved by hybridoma methodology – which typically would have yielded a few useful clones from each mouse. By developing and applying single cell methods we are now able to recover 100-200 antigen specific B cells from each immunised mouse, which means the mouse numbers required for immunizations are much lower than they were previously.

Why can’t you use animals that are less sentient?

The immune systems require several months to develop a robust immune response against a target. The immune system is not mature at an earlier stage (immature life stage).
Lower organisms can’t be genetically engineered to the extent possible with mice, the mechanisms of an immune response are different and the structure of antibodies are also different, so other species can’t be used.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I don’t anticipate many refinements to the transgenic methods as these are already very standardised and efficient.
Advances in single cell sequencing technologies should lead to increases in the amount of data that we can obtain from a single mouse. For example, more complete information on the B cell responses to an antigen will enable us to use fewer mice and will also allow us refine immunisation methods.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I use the guidance on the NC3Rs web site https://www.nc3rs.org.uk and the IMPC website https://www.mousephenotype.org.
I also use documents prepared by the local establishment. Animal experimentation conducted under this project licence will comply with the guidelines in this document except where permission for a specific procedure or procedures vary from it.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I periodically check the NC3R's website which has many excellent SoPs and videos. I'm also a member of ISTT and its members are frequently communicating advances in the field and their experience implementing these.

Home Office

NON-TECHNICAL SUMMARY

120. Microbe and body rhythm influences on the development of type 1 diabetes

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Immunology, Autoimmune diabetes, Circadian rhythms, Metabolism, Infection

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to understand the effect of daily body rhythms on bacteria that are found in the gut, and how this influences cells of the immune system (CD8 T cells) to 1. attack their target cell in the pancreas (beta cells) in type 1 diabetes, at different times of day, and 2. alter how well immune cells deal with infection.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There is currently no therapy available for preventing development of Type 1 diabetes, which is an autoimmune disease whereby the immune system of the body attacks insulin-producing beta cells of the pancreas. Insulin injections, required several times a day, are a life-saving/life-maintaining treatment, but many people find it very difficult to maintain good blood glucose control and may develop long-term complications. In this work, we aim to understand how body rhythms that regulate hormones and metabolism, as well as the immune system, work together with the bacteria that live within the gut. This will help us develop new knowledge about development of therapy based on regulating the immune system, and potentially, how best to give this therapy.

What outputs do you think you will see at the end of this project?

This work will generate new information about the way the daily rhythms of the body can influence the bacteria that live within us, the way the immune system works, and add to information about how both of these interact with the insulin-producing cells of the pancreas. The work will be published in scientific journals.

Who or what will benefit from these outputs, and how?

Other scientists will benefit in the short term, as it will add to information that should enable us to design better treatment, with a time scale of 5 years. Ultimately, as we understand the science better, and new treatments for type 1 diabetes are developed, this could benefit people who have type 1 diabetes, as well as those who may be protected from developing disease. The benefits could be seen in 5 to 10 years. In addition, understanding the way bacteria and the insulin-producing cells of the pancreas interact could also benefit people with type 2 diabetes, and provide basic information that may lead to further studies. Studies of the immune daily rhythms may help people with both type 1 and type 2 diabetes, as they have relevance for why people with diabetes may be more susceptible to infection.

How will you look to maximise the outputs of this work?

The outputs will be maximised by collaboration with other investigators, as well as people from allied fields of research who will find the principles that underlie the effects of the daily rhythm on the immune system, the hormonal system and the gut bacteria of importance to their work. We will disseminate the new knowledge to scientists at scientific meetings, the community of people who have type 1 diabetes, as well as the general public through public engagement activities. We will publish the work in scientific journals.

Species and numbers of animals expected to be used

- Mice: 15000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse is the species of choice here because of the availability of strains and the immunological reagents, which allow investigation of development of immune T and B cells, together with the ability to track immune cells. They are also important in the study of responses that are similar to human immune responses.
Many of the mice used do not have any obvious abnormality that affects their day to day existence. Some mice are known to develop diabetes and we need to use these as the model of human type 1 diabetes, as our aims in this project are to study disease processes leading to diabetes. These will enable us to design treatment in the future.

We will study mice from neonates to aged mice. The neonates, and juvenile mice are studied because it is known from our work and those of others that influences early in life affect future development of diabetes. We study adult and aged mice, as they are nearer to development of diabetes, and it is important to study the disease processes, both early as well as later in life. Most mice will be studied before development of disease or within a short period after diagnosis. Some mice have a deficient immune system, and the use of these is necessary to allow us to study the cells recognising insulin and other self protein targets, without interference from other immune cells.

Typically, what will be done to an animal used in your project?

We have a number of different protocols in our project, and mice will have limited number of procedures as part of these protocols.

Initially, we will study mice that have one of either of the following conditions, prior to studying gut permeability and glucose tolerance:

1. Changes to light cycles
2. Changes to diet type
3. Changes to access to food (food restrictions)
4. Exposure to antibiotics.
5. Immunizations
6. Infection
7. Imaging

Investigation of insulin tolerance will only be conducted if the glucose tolerance results indicate a difference between groups.

Once we have conducted these initial studies, and they have been shown to be safe, we will then study combinations of conditions 1-7, the combinations of which will be determined by the data obtained. These combination studies will involve studies on mice receiving 2 conditions, the data from which will then generate avenues of exploration for a new set of mice to receive 3 conditions and so forth. This method will allow us to establish that the combined conditions are safe and provide important data to further focus the specific research question, minimizing unnecessary animal use.

What are the expected impacts and/or adverse effects for the animals during your project?

Up to 20% of the mice in the project as a whole may develop diabetes. They drink more and urinate more. The cages require more frequent changing. If they are not treated with insulin, they may start to lose weight. We would terminate the experiments within 2 weeks of developing diabetes, before the mice become unwell from diabetes.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most of the mice will have mild severity. If they develop diabetes, this would be considered moderate.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We will study aspects of development, where possible, in vitro. However, we cannot investigate interaction of the circadian rhythm, the gut bacteria and effects on the immune system to cause diabetes in people. We are unable to manipulate the conditions in humans, to fully dissect the importance of different components. We will use cells that arise under altered selection conditions in vivo, to test the influence on diabetes development. This has to be done in vivo as diabetes arises as a complex process that involves numerous cell interactions and the metabolic state, which cannot be recreated in vitro. Diabetes occurs because cells migrate to the pancreas, enter the pancreas and attack the insulin producing cells. The influences within the whole mouse that affect all these processes cannot be studied in tissue culture.

Which non-animal alternatives did you consider for use in this project?

Where possible we will study effects of changing conditions in tissue culture. We will also study these influences in humans. However, in people, we cannot investigate interaction of the circadian rhythm, the gut bacteria and effects on the immune system, which come together to cause diabetes .
Why were they not suitable?

We are unable to manipulate the different components leading to diabetes, with the appropriate controls, in humans.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimated numbers of animals is based on the known characteristics of the breeding of the animals, the need to carry out complex breeding, the number of experiments required in each protocol and the numbers estimated to be required for each set of experiments to give valid and meaningful results.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used the NC3R Experimental Design Assistant and have been guided by the use of power calculations, and the requirement to carry out pilot experiments, where there is no previous information about the expected outcomes of experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies to assist our final experimental design where there is no previous information about the expected outcomes of experiments. We have developed protocols that reduces the number of donors required for experiments by optimizing the numbers of cells for experiments. We have consulted a statistician in planning our experiments to use the minimum number of mice to give us sufficient information based on statistical considerations. Where possible non-invasive imaging will be used to follow development of diabetes, rather than multiple animals. We will use the most efficient breeding protocols that we have designed based on many years of experience with the NOD mouse model.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse is the species of choice here because of the availability of strains and the immunological reagents, which allow investigation of development of immune T and B cells, together with the ability to track immune cells. They are also important in the study of responses that are similar to human immune responses.

Many of the mice used do not have any obvious abnormality that affects their day to day existence. Some mice are known to develop diabetes and, apart from mice that will be treated with insulin, they will be studied before development of disease or within a short period after diagnosis. There are mice in which the immune system is deficient which are necessary to allow us to study the cells recognising insulin and other self-protein targets without interference from other immune cells. All mice will be housed in isolators, individually ventilated racks or filter framed cages to reduce the risk from infection.

Why can’t you use animals that are less sentient?

We cannot use animals at a different life stage, as we study diabetes in a model that has close resemblance to type 1 diabetes in humans. Species that are less sentient do not develop diabetes. Our experiments require day to day observation of complex physiology and immunology, and cannot be done on terminally anaesthetised

animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will monitor all mice for the development of diabetes. Many of the mice used do not have any obvious abnormality that affects their day to day existence. The mice that can develop diabetes will be studied before development of disease or within a short period after diagnosis (unless treated with insulin). There are mice in which the immune system is deficient which are necessary to allow us to study the cells recognising insulin and other self antigens without interference from other immune cells. All mice will be housed in isolators, individually ventilated racks or filter framed cages to reduce the risk from infection.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will use the PREPARE and ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will consult the NC3Rs website regularly, and at least monthly, to remain up to date on advances in the 3Rs. We will subscribe to the NC3Rs newsletter. We also remain aware of current literature that is published.

Home Office

NON-TECHNICAL SUMMARY

121. Modelling organ fibrosis and cancer in mice

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

fibrosis, cancer, therapy, inflammation, chronic disease

Animal types	Life stages
Mice	adult, pregnant, neonate, juvenile, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this study is to advance our understanding of how organ fibrosis (scar formation in damaged tissue) and liver cancer develops as well as identify new drug targets and test new medicines for the treatment of these diseases.

A retrospective assessment of these aims will be due by 01 June 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is estimated that tissue fibrosis, which can affect any organ in the body, contributes to up to 45% of deaths in the developed world. The number of people diagnosed with organ fibrosis, particularly liver fibrosis, are increasing every year. Currently, only two drugs are licensed to treat fibrosis in a rare lung disease, however, there are no medicines approved to treat other lung disease or fibrosis in other organs.
Liver fibrosis is also a risk factor for developing liver cancer, and with Sorafenib, the only drug used to treat advanced liver cancer, only extends life expectancy by 3 months. Therefore, there is an urgent need to better understand the disease and develop improved liver cancer models for drug testing.

What outputs do you think you will see at the end of this project?

Work conducted under this project licence will generate data and new knowledge that will help advance our understanding of how organ fibrosis develops and what changes at the level of molecules and cells make the disease get worse. Our work will help advance knowledge the disease process and identify new drugs or drugable targets, which limit scar formation in damaged organs or prevent liver cancer.
We aim to identify biomarkers of disease (e.g. proteins or DNA in the blood that are released from the damaged liver) or imaging tools to help assess how advanced the scarring or cancer is or provide information if a drug is working.
Our work will be presented at scientific meetings and published in research papers to share knowledge gained with the wider scientific community and help advance knowledge in the field, ultimately for patient benefit.

Who or what will benefit from these outputs, and how?

In the short-term, scientists in both academia and industry will benefit from the discoveries generated under this program of work. This could be due to the development of new research tools, experimental approaches or identification of new pathways which when targeted yields therapeutic benefit.
Ultimately, the long term aims is to benefit patients either through development of new diagnostics (biomarkers or imaging tools) or new treatment strategies.

How will you look to maximise the outputs of this work?

By presenting our discoveries and national and international scientific meetings, publishing our research discoveries and through collaboration with academics or the pharmaceutical industry, we will be able to

maximise the impact of knowledge gained under this program of work.
Wherever possible we collaborate with others to share tissue samples, lines or provide training in methods through collaborative research or participation in workshops.

Species and numbers of animals expected to be used

- Mice: Up to 50,000 breeding and 39,100 experimental animals

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Tissue fibrosis and liver cancer develops over many weeks/years therefore adult mice are used.
To study disease biology and ask how a protein effects the disease process we need to use genetically modified mice which do not express that protein or express a modified (or mutant) form of the protein that is found in patients, identified in genetic studies as increasing the risk to develop that disease or a more aggressive form of the disease. Genetically modified mice are readily available therefore mice provide an optimal system to study the disease. Additionally the research tools/reagents needed to investigate disease mechanisms and test therapies are available and are able to be translated to higher mammals.

Typically, what will be done to an animal used in your project?

Chemical models of liver or lung fibrosis have been refined for many years and are very predictable models, therefore we know what the disease stage animals have reached at any given time point. We have lots of experience running drug studies in these models, therefore we know exactly when to give drugs and for how long to administer them. In the liver disease model mice receive bi-weekly injections of the chemical into their belly, whist in the lung model a chemical is inhaled into the lung. For some liver fibrosis models chemicals may be placed in the drinking water. We also use chemicals to induce liver cancer, where a mouse receives a single injection in the belly and cancer develops over many weeks.
Surgical models will be used to study liver disease, kidney disease, liver cancer or assess liver regrowth after partial removal of the organ. Pain relief is always given as needed. The liver disease models accurately mimic's a different type of liver fibrosis, where the bile duct is tied preventing flow or bile in to the intestine, bile spills into the liver and causes damage, this mimics a disease called cholestatic liver disease. To study liver growth, up to 70% of the liver is removed and then allowed to regrow over a period of 5 days (when normal mass is restored). Liver cancer cells are injected into the liver to study liver cancer and then test drugs. Cancers may develop over a few weeks. To induce kidney disease the ureter (a tube connecting the kidney to the bladder) is surgically tied to prevent urine flowing from the kidney to bladder, which causes kidney injury and fibrosis.
To model diet induced liver disease we will feed mice a modified diet (e.g. high fat) either with or without sugar water (comparable to coke). These animals gain weight and develop features of fat induced liver disease. Other dietary models include the methionine and choline deficient (MCD) diet model we expect mice to lose weight but develop fat induced liver disease.
Skin inflammation (redness and swelling caused by white blood cells) and scarring will be induced in the mice by either giving agents, which irritate the skin or chemicals that cause scarring. Punching two small holes in the skin and then watching them heal will be used to study how skin wounds heal. Pain relief is given as required.
Liver cell death will be promoted by giving mice chemicals or drugs at toxic doses (e.g. paracetamol) or inflammatory molecules, doses may be lethal or sub-lethal and mice will recover after a short period of feeling unwell.

Bone marrow chimera (a bone marrow transplant): normal or genetically modified (GM) mice will be irradiated to remove the white blood cells and then given new immune cells from a donor mouse of a different background e.g. normal in to GM.
All of our disease models are used to test therapies for that type of chronic disease or cancer.
In these models we may wish to perform non-invasive imaging whilst the animals are asleep, assess well-being using behavioural test or measure blood pressure. We may take blood samples to assess disease stage or drug metabolism. In disease models we might perform glucose tolerance tests.

What are the expected impacts and/or adverse effects for the animals during your project?

In our mouse models, mice may show signs of sickness e.g. hunched posture, diarrhoea, ruffled fur, look pale or feel cold. Supportive care and pain relief will be given and if the clinical condition does not improve within 24h mice will be humanely killed.
Surgical models: adverse effects due to surgical complications may include bleeding. Mice were the bile duct has been tied, occasionally become jaundice (skin becomes a yellow colour) or get swelling in the belly. Supportive care such as fluids, soaked diet and a warm environment will be provided. Pain relief is always given in surgical models and animals are carefully monitored by the surgeon and an assistant prior to and during the surgery to ensure that animals are sufficiently anaesthetised throughout the procedure.
In the dietary models most mice will gain weight, but not to a point where mobility is impaired, but in the methionine and choline deficient (MCD) diet model we expect the mice to lose weight.
Liver cancer model, mice will develop liver cancer over a period of up to 60 weeks but this will not result in liver failure.
Liver cell death caused by toxic chemicals or drugs will be either lethal where mice are humanely killed when very sick and the liver starts to fails or sub-lethal where mice will fully recover.
Lung fibrosis; mice will develop lung disease over a period of up 28 days and can lose weight during the first week of the models but regain weight after this time.
Skin fibrosis; the skin may become red (inflammation) and the skin will become thicker. Skin wound healing; the wounds created by “skin punching” are superficial and therefore do not bleed. These models last up to 28 days.
Kidney fibrosis; mice will develop kidney disease over a period of up 18 days and can lose weight during the first week of the models but regain weight after this time.
Heart fibrosis will be induced by increasing blood pressure, which stresses the heart by chemical infusion or by surgically restricting the aorta. Mice can lose weight during the first week of the models but regain weight after this time.

Bone marrow chimera: Mice will always receive replacement bone marrow after irradiation, however, they may be sick and lose weight due to the effects of the irradiation but will recover once the immune system has been replaced. Rarely the transplantation of the bone marrow may fail, if this occurs (animals fail to regain weight) then the animal will be humanely killed.
Imaging: mice may experience a small weight loss post imaging but this should recover within a few days.
Therapy, we do not anticipate therapies will cause significant adverse effects.
In all models Pain relief is given when needed.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Only mice will be used in this program of work. We estimate that ~80% animals used will in procedures will be mild including breeding mice to maintain a colony, for experiments and to take tissue or perform mild models of disease such as acute liver injury or dietary models of disease. Cell isolation from liver tissue in non-recovery procedures comprise ~1% of experiments. Approximately 16% of animals will be of moderate severity, whilst up to 3% may fall into the severe category.
What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 01 June 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are studying complex diseases which develop and heal over many years.
Tissue fibrosis, a term which describes scar formation in an organ as a result of tissue damage is caused by many different cells in the organ changing their behaviour to try and heal the wound. If the damage is only small or limited to one insult, then the scar will dissolve. However, when the organ is repeatedly damaged over and over again, the scars persist and then reduce the ability of the organ to perform normal daily tasks. When disease is very advanced an organ can fail. Fibrosis occurs not only as a consequence of damage to the tissue but also through recruitment of white blood cells to the injured tissue, therefore signals from outside the affected organ are an important of the disease process.
Cancer, is also a complex disease, which involves many different cell types but is more likely to develop in a diseased or fibrotic organ e.g. liver disease.
This research will identify proteins which either cause or limit fibrosis or cancer. To prove that they do disease models may be performed in mice which are "genetically engineered" and lack the protein of interest.
Alternatively, we may wish to test drugs which we believe will limit tissue fibrosis or cancer growth.
For these reasons we need to perform some of our research and drug testing in animals.

Which non-animal alternatives did you consider for use in this project?

We routinely use human cells in culture (including cell lines) to understand and model the biological processes involved in causing tissue fibrosis and cancer or to perform drug testing.
We have started testing/developing 3D cell culture models as an alternative method for drug screening and understanding the biology of scar cells or cancer cells.
We have recently optimised a new technology to culture very thin slices of tissue from human liver, lung and kidney to model fibrotic disease and test scar-limiting drugs. We are now developing methods to model other fibrotic diseases. These technical advances will help minimise use.

Why were they not suitable?

Whilst these are useful tools, there are limitations of cell cultures systems, these include;

1. cells grown in petri-dishes sit on plastic, which is much stiffer than where they reside in the body. The increased stiffness can change their behaviour and they become "super activated" or fail to do the job they would in the body. These abnormal behaviours could lead to the identification of non-relevant pathways or fail to predict drugs which are likely to be ineffective in the disease.

2. Organ fibrosis development and resolution is regulated by many different types of cells communicating with each other within the damaged organ as well as through communication with white blood cells and receiving signals from other organs. Recreating all of these internal and external organ damage signals

is difficult to model in culture.
3. Using genetic engineered mice will help advance our understanding of the disease to help identify new targets for drug discovery.

Such systems are extremely useful for aiding our basic knowledge and for initial drug screening, and we use these systems to reduce animal use.
A retrospective assessment of replacement will be due by 01 June 2026

The PPL holder will be required to disclose:
- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Yes, we have used knowledge from previous studies to mathematically calculate the minimum number of animals needed in each group to generate data which allows us to answer our scientific questions. By doing this we can minimise use but be confident that the differences in a scientific measurement between two groups is meaningful and has not been obtained by chance.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have taken multiple approaches to ensure that we use the minimum number of animals for research purposes.
We have performed audits of our previous research studies and assessed research plans of current projects to predict use under this project.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Statistical analysis is performed to determine the minimum numbers of animals need to generate biologically meaningful data. If this is not possible then pilot studies are performed to reduce numbers and inform future studies going forward.
We employ efficient breeding strategies and where possible we use both male and female mice to minimise numbers of animals used.
Tissue is regularly shared between projects to maximise outputs from animal procedures and minimise numbers of animals used.
Wherever possible, we will use human tissue/cells or cell culture systems to replace animal models of organ

fibrosis and cancer. The group have accumulated archival tissue banks of frozen and formalin fixed tissues from our previous models and human normal and diseased tissues. These samples are used in multiple on-going projects to minimise the number of animal disease models used.

A retrospective assessment of reduction will be due by 01 June 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

All of the disease models chosen are the lowest severity model that can be used to answer our research questions.
Accumulation of scar tissue in response to organ damage, namely fibrosis, can affect any organ in the body and is caused by many different types of injury. Scarred organs are also at a higher risk of developing cancer. Therefore to understand how this disease develops, determine if this process is common to all organs and test new therapies, we need to perform different models of tissue injury.

For example, chemical models of liver fibrosis or lung has been refined for many years and is a very predictable model, therefore we can predict the disease stage at any given time point. We have lots of experience running drug studies in these models, therefore we know exactly when to give drugs and for how long. In the liver disease model mice receive bi-weekly injections of the chemical into their belly, whist in the lung model a chemical is inhaled into the lung. We also use chemicals to induce liver cancer, where a mouse receives a single injection in the belly and cancer develops over many weeks.
Surgical models will be used to study liver disease, liver cancer, kidney disease or assess liver regrowth after partial removal of the organ. Pain relief is always given as needed. The liver disease models accurately mimics a different type of liver fibrosis and is used to test therapies for that type of liver disease. To study liver growth, up to 70% of the liver is removed and then allowed to re-grow over a period of 5 days (when normal mass is restored). Liver cancer cells are injected into the liver to model liver cancer. To induce kidney disease the ureter is surgically tied to prevent urine flowing from the kidney to bladder, which causes kidney injury and fibrosis.
To model diet induced liver disease we will feed mice a modified diet (e.g. high fat) either with or without sugar water (comparable to coke). These animals gain weight and develop features of fat induced liver disease.
The most refined model is always used.

Why can’t you use animals that are less sentient?

Organs contain lots of different types of cells and these cells all talk to each other to ensure that the organ does its job properly. When an organ is damaged, all of the different cell types are needed to help heal the injury. White blood cells also enter the organ at the site of injury to help clean up the damage and heal the wound. However, when organs are repeatedly damaged this healing process becomes abnormal and fibrosis occurs.

Fibrotic diseases in the liver, lung, skin, kidney, heart and liver are complex processes that develop many weeks/years. Fibrotic organs are also at a higher risk of developing cancer.
Because of the complex nature of the disease process it is not possible to recreate this using cells in culture dishes, therefore we need to study disease progression and test medicines in the whole animal.

Genetically modified mice, which lack a protein which is thought to be important in the development of tissue fibrosis are used to help understand the disease process better but also to help identify new drug targets to treat organ fibrosis and cancer.
Fibrosis is an inflammatory driven disease. Fish and insects (e.g. flies) lack the same broad range of immune cells found in humans, which means that there are differences between fish and mammals that could affect the disease biology. Some genes are not conserved between these species and mammals therefore some of the disease mechanisms may not be the same. Therefore drugs that target those cells or disease pathways may not work in these systems due to fundamental differences in the biology of the species compared to humans.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals, regardless of disease model are checked regularly and supportive care is readily provided to minimise distress or suffering and improve animal welfare.
For surgical models we use good surgical techniques and operating theatres/equipment to minimise the risk of infection. The bile duct ligation model is a surgical model of liver fibrosis. In this model mice receive pain relief and a high level of post-operative care including soaked diet, a warm environment and fluids as required to minimize stress and suffering. By working with the vet team and animal behaviour/welfare scientists we have developed a clinical scoring system to help assess the animal's well-being and level of disease.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

For all models and optional procedures good practice guides will be used to help refine the model as described in the ARRIVE guidelines and Laboratory animals special article 2015, 49 (s1).
LASA Working Party guidelines on assessment and control of severity will be used throughout the project to determine if any animal is suffering distress.
Workman et al in 2010. British Journal of Cancer (2010) 102, 1555–1577 NCRI guidelines will be used to perform and monitor liver cancer studies.
Should distress occur immediate actions as described in the individual protocols would be taken to reduce an animals suffering.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

There are many sources in which provide information regarding 3Rs advances, these include; the NC3Rs website, NC3Rs seminars/events and emails, scientific publications and published guidelines as well as continued professional development e.g. local seminars, regular communication with the NACWO and veterinary team and academic collaboration with the welfare group.

As information on welfare or technical improvements, alternative less severe models or new non-animal model systems becomes available an appropriate strategy within the research group and veterinary teams will be implemented to ensure that animal use and suffering is minimised. This will include testing new models (animal or non-animal) and modifying procedures. **A retrospective assessment of refinement will be due by 01 June 2026**

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

122. Models to advance understanding and treatment of lethal children's brain tumours

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

paediatric, cancer, brain tumour, precision therapy

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Brain tumours are the leading cause of cancer-related death in children, of which the most lethal type is commonly referred to as paediatric high-grade glioma (pHGG).The overall aim of this project is to advance understanding of the origins, underlying biology and treatment of pHGGs, for which currently there are no effective treatments that improve survival for patients. With greater understanding of how pHGGs develop and propagate, more precise therapies tailored to the unique biology of these tumours can be identified.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Paediatric high-grade gliomas (pHGGs) are incurable and a leading cause of cancer-related death in children, affecting approximately 70-100 children in the UK every year. At present there are no effective treatments for this universally fatal disease. Current standard treatments are broad-acting and invasive, involving debilitating chemotherapy, radiation and brain surgery, and ultimately do not improve survival. In fact, often there are also unintended effects on the still-maturing brain, which negatively impacts quality of life in the longer term for children receiving treatment. This dismal prognosis has remained unchanged for decades with less than 5% of patients surviving 5 years post-diagnosis.
Our aim is to improve the outlook for pHGGs by developing more precise therapies based on their unique biology. The accurate recapitulation of this disease using state-of-the-art animal modelling is crucial for this, to further our understanding of the biology of this disease and to develop newer, more effective precision therapies. With these models we hope to understand how these tumours form and propagate, discover what makes them different from normal brain cells, and find out how these differences play out in the tissue micro-environment of the brain; perhaps by recruiting other normal brain cells in support of tumour growth or enabling tumour cell evasion from immune surveillance. This information can only be gained using animal models of disease, and is important for developing the next generation of cancer treatments.
Specifically, this work guarantees new insights into how co-operating genetic mutations induce and maintain paediatric high-grade gliomas. The project also aims to reveal distinct therapeutic vulnerabilities in different glioma sub-types.

What outputs do you think you will see at the end of this project?

This research focuses on the mechanisms driving the development of children’s brain tumours. We expect to make advancements in our understanding of both fundamental and therapeutically relevant aspects of brain tumour biology. Therefore, the outputs will include new knowledge and publications as well as potentially, products such as novel diagnostic tools and/or therapies. These outputs will be of interest to academic researchers, clinician scientists, healthcare practitioners, drug development experts and pharmaceutical companies. However, the main impact of this research in the longer term will be on paediatric brain tumour patients and their families, as well as the NHS and social services.

Who or what will benefit from these outputs, and how?

Since current treatments for paediatric brain tumour patients produce debilitating and often life-long side-effects, the novel, potentially more effective treatments that this research aims to develop should be less damaging and produce fewer complications in patients. This should alleviate some of the costs associated with long-term care for the NHS, social services and families caring for patients.
In the short-to-medium term, scientists working on improving our understanding of paediatric brain tumours and others aiming to develop new treatment strategies for this cancer will benefit the most from the tools and data this proposal will generate. This is because the models and molecular dependencies this research will uncover

will be most relevant to their work. Academic researchers interested in brain development, stem cells and cancer will also benefit from the new modelling techniques we will develop. These can be used for manipulating neural stem cells in mice, to gain insights into brain development, stem cell function and cancer.
In the longer term, healthcare practitioners such as those designing clinical trials, drug developers in academia or industry and pharmaceutical companies can benefit from this research. They will be able to use these results to identify and refine patient populations they wish to target therapeutically or diagnostically, to improve the design of candidate therapies, or to evaluate the efficacy of new treatments.
Regarding the contribution of this research to the economic competitiveness of the UK, this work may identify candidate diagnostic markers and novel therapies with commercial potential. These commercial opportunities can add to the UK's already considerable competitiveness in the health and life sciences sector.

How will you look to maximise the outputs of this work?

Sharing data and communicating our progress to scientific, clinical, industry and lay audiences, and ensuring that this information is freely available, forms the basis for maximising the output of this work. The strategies outlined below will communicate our work to academic researchers, clinician scientists, healthcare practitioners, drug developers and pharmaceutical companies. This will ensure translation of novel diagnostic tools and experimental therapies to the clinic, so that patients' lives improve. The research outlined in this proposal will impact health and well-being, economic competitiveness, and alleviate strain on health and social services here in the UK.

Academia and Industry

1) Collaboration with Drug Development Teams – The research proposed in this application will identify drug targets for brain tumours, which will then be passed on to our collaborators for drug development and partnering with pharma. Eventually we hope to spearhead clinical trials and translate new drugs into the clinic.

2) Peer-reviewed Open Access Publications – All scientific results, including experimental diagnostic markers, therapeutic candidates and genetic dependencies specific to each tumour type will be published in peer-reviewed journals. All articles will be open access to ensure they can be widely disseminated. I will also prepare accurate press-releases publicising each publication.

3) Scientific Conferences – I will regularly present the results of this research at multiple national and international conferences. I will also share ongoing work "pre-publication" to receive vital feedback from the scientific and healthcare community and to guide and publicise my work. This will take the form of "work-in-progress" seminars and posting to preprint servers.

4) Data Sharing – The proposed project will generate a significant amount of data carried out on cells harvested from tumours in mice. These tumour cells will possess different combinations of mutations and will be screened with different drugs and gene editing technologies. To make this complex and rich source of information available to the widest possible audience, all sequencing and screening datasets generated will be deposited to publicly available online databases. Molecular biology reagents enabling the generation of tumour models in mice will be made available publicly as well, for dissemination to the wider brain tumour research community. Tumour cell lines generated during the course of this study will be deposited to publicly accessible cell repositories.

General Public

1) Public Engagement Seminars – I believe strongly in public engagement and science communication, and will participate in public seminars throughout the course of this project.

2) Scientific Media – I will actively engage with print and online media following publication of my work and will commission creative content to describe the results in general, visually arresting and moving ways. I believe this strategy will expand the impact of the work immeasurably, enabling people to see past the complexity of the disease mechanisms and appreciate the progress on cancer that the work will represent. I believe this strategy will help engender a scientifically literate and well-informed society.

3) Summer School – I will participate in an annual summer school event. The aim of this event will be to increase understanding of the specific challenges faced by brain tumour patients, to identify the pinch points in the clinical care of these patients, and reveal blind spots in the focus of biomedical researchers. Ultimately, the aim is to fast-track the development of new, more effective treatments to improve outcomes for this disease.

Species and numbers of animals expected to be used

- Mice: 7290

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Brain tumours cause the most deaths due to cancer in children. The prognosis for the most lethal type of children's brain tumour, called paediatric high-grade glioma (pHGG), is particularly dismal and has remained unchanged for decades. Currently in the clinic, pHGGs are treated exactly the same as adult brain tumours, even though children's tumours have completely different causes and underlying mechanisms. These conventional treatments also have a significant negative impact on quality of life, as children's brains are still growing and maturing when these harsh and inexact treatments must begin. To improve the outlook for pHGGs and develop new therapies that are kinder, more effective and more precise, we must understand more about their unique biology.
Mice are essential for the proposed research. Cells in a Petri dish cannot fully replicate the diversity and complex biology of the mammalian central nervous system. Moreover, invertebrate or nonmammalian models cannot reproduce many biological and physiological aspects of human brain tumours. Mice share a similar genetics, development and central nervous system anatomy with humans, and glioma models in mice display many similarities to human brain tumours. Of the commonly used model organisms, the biology of the brain, the brain regenerating cells (stem cells), the immune system and the response to chemoradiotherapy in mice are the most analogous to human. Therefore, to make clinically relevant discoveries and develop more effective therapies for pHGGs, experimental work using mice is essential.
Our previous work demonstrated that in order to model children's brain tumours such as pHGGs in mice, mutations have to be delivered into brain stem cells (also called neural stem cells or NSCs) either during gestation (i.e. embryonic development), or very soon after birth, in neonatal pups. When we delivered mutations later, in older animals, tumours failed to develop. The reason for this selectivity— why children's brain tumours only arise from mutations occurring at the earliest stages of life— is because children's brain tumours arise from mutations within NSCs that occur while the brain is being formed. Since the brain is formed during gestation and the first few years of life (after which the brain is relatively stable and there aren't many more cells being constantly added) these mutations in NSCs must enact their first cancer-causing effects in utero, before birth, or very soon after. This is in stark contrast to adult cancers that arise from mutations in adult stem cells. These mutations in adults require a lot longer to enact their cancer-causing effects and are also acquired later in life, in part due to lifestyle choices (smoking, obesity). In short, paediatric cancers are fundamentally different from adult cancers: arising in different stem cells and through different mutations. This causes paediatric cancer causing mutations to act much earlier in life (often before birth itself) to induce tumour development. This is the rationale for delivering mutations into embryonic and/or neonatal mice to model pHGGs in this project. We introduce the paediatric glioma mutations in mice at a similar life stage to when they occur in humans, i.e. during gestation. Then brain tumours develop in mice, just as they do in humans, thereby creating a mouse model that accurately represents the human disease. These models can then serve as a good system from which to infer knowledge about the human disease and to identify and test new therapies.
pHGGs are highly heterogeneous cancers – with many different co-occurring mutations and types of cells found

within them. Genetic sequencing has revealed that they comprise several distinct tumour subgroups or types: based on the different constellations of mutations they carry and the particular region of the brain in which they are found. However, the relevance of many of these extra mutations that are used to define these distinct tumour types is unknown. Furthermore, it remains unknown whether these extra mutations make some tumours more sensitive to certain drugs. In this proposal, I aim to understand precisely how different co-occurring mutations support tumour growth, with the goal of using this information to undo these tumours.
To do this, we will induce different types of pHGG tumours in mice, each type driven by a different combination of mutations. I propose using this approach to test the effects of these extra mutations, to find gene level changes that help drive these different types of tumours. In the process we will also discover what intrinsically makes these tumours distinct from one another. These results will then be confirmed in human tumour cells. In this way, research in mice will be used to find the proverbial needles in the haystack, and then the most promising candidates (aka gene level changes) will be validated in human cells.
Therefore, I hypothesise that co-occurring mutations used to group tumours into different types can be functionally evaluated using mouse models to reveal clinically tractable vulnerabilities.
In this project, we will:

1. Functionally evaluate co-occurring mutations and develop mouse models of highly aggressive children's brain tumours.

2. Identify the genes and gene level changes important for the growth of different types of tumours in mice.

3. Validate these genes and gene level changes in human tumour cells.

I also propose using this approach to test the effects of the different mutations in unprecedented detail, to find out how they alter the environment of the tumour. I anticipate that each of the mutations has a role in helping tumours grow, by influencing in turn the roles played by different types of cells in the tumour and how they interact with their neighbouring cells, immune cells and blood vessels. I will measure these mutation-driven changes in the tumour ecosystem by analysing precisely how the function, and relative amounts, of different types of cells is altered by different mutations. This will help us understand how co-occurring mutations change relationships between cells to allow tumours to grow, and how this differs between tumour types. This information will help us develop more precise, targeted therapies. This environment-centric approach is necessary to identify how genetic heterogeneity, i.e. the many different co-occurring mutations found in these tumours, helps support tumour growth.
The ultimate goal of this proposal is to aid in the development of a “precision” patient- and mutation matched approach to treating this devastating disease. Therapeutic opportunities that are revealed through the experiments proposed here, if validated in the clinic, will represent the most appropriate treatment for children with pHGGs carrying similar combinations of mutations.

Typically, what will be done to an animal used in your project?

For tumour induction, surgery will be performed on pregnant females to deliver DNA/virus/cells into the brains of embryos midway through gestation. Alternatively, DNA/virus/cell injections will be performed on pups no more than a day old, in which case surgery will not be required. Following surgery and/or injections, mice will develop normally while brain tumours develop. As soon as they begin to display symptoms of brain tumours, usually >4 months later, they will be humanely killed and their brain and tumour tissues will be harvested for our experiments.
Therefore, the outcome from the surgeries and injections are the mice that develop brain tumours over time. These tumours in mice will model lethal and incurable brain tumours occurring in children and young adults. Mice will be monitored using weekly imaging until onset of symptoms that necessitate humane killing. Tissue collected following killing will be used to establish cell cultures or for microscopy. The primary outcome of this work will be data describing increases or decreases in the time it takes for tumours to form and symptoms to develop, the appearance of the tumours and their aggressiveness in mice. The secondary outcomes will be the changes we can observe in Petri dishes, in terms of the ability of the harvested tumour cells to grow over time,

their ability to infiltrate and invade other areas, and other measures of tumour cell activity.
Optionally, tumour-bearing mice will be treated with labelling, imaging or chemotherapeutic agents, and may in addition be treated with focal, targeted radiotherapy. Labelling, imaging and chemotherapeutic agents may be delivered orally or by injection. Focal irradiation will be delivered in some cases together with chemotherapy. The use of focused radiation greatly reduces the adverse effects associated with whole-body or whole-cranial irradiation. These procedures will allow us to replicate in mice how brain tumour patients are treated in the clinic, and to test experimental therapies.
All mice will be housed, bred and subjected to listed procedures according to institutional, Animal Welfare and Ethical Review Body (AWERB) and UK Home Office guidelines. Every attempt will be made to follow the NC3Rs guidelines to reduce, refine and replace the number of mice. All work will be carried out with veterinary and animal husbandry staff oversight and under regulated procedures of mild to moderate severity. The utmost efforts will made to minimise pain, suffering and undue distress. The evaluation criteria for humane killing and tissue collection will be based on observation of neurological impact. Mice will be monitored daily for neurological symptoms, i.e. weight loss, lethargy, seizure prevalence, immobility and changes in gait, grooming or behaviour. As soon as neurological symptoms are observed, in collaboration with animal husbandry and veterinary staff, a decision will be made regarding humane killing, and tissue will be collected for research purposes.

What are the expected impacts and/or adverse effects for the animals during your project?

The appearance of any one of the following adverse effects will trigger humane killing of the animal to prevent undue suffering. The appearance of any one of these adverse effects will also mark the experimental endpoint, following which tissue will be harvested for experimental purposes. These effects are expected between 1 and 18 months following administration of the procedures listed in this project. These effects will arise due to the presence of brain tumours, and may include:
-Loss of appetite and weight, which is a common symptom of brain tumours and chemo/radiotherapy. This will be monitored and if weight loss is greater than 15%, or 20% for chemo/radiotherapy-treated animals, they will be killed to prevent undue suffering.
-The presence of brain tumours can cause problems with walking (gait problems, wobbling, or limb dragging) . If this occurs, animals will be killed to prevent undue suffering.
-The presence of brain tumours can sometimes cause the head to become tilted . If this occurs, animals will be killed to prevent undue suffering.
-The presence of brain tumours can sometimes cause seizures to occur. If this occurs, animals will be killed to prevent undue suffering.
-The presence of brain tumours can sometimes cause excessive lethargy. If this occurs, animals will be killed to prevent undue suffering.
-The presence of brain tumours can sometimes cause fluid to build up inside the brain (hydrocephalus). If this occurs, animals will be killed to prevent undue suffering.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severity limit is: Moderate
70% of the animals will experience procedures of Moderate severity (animals on Protocols 2 and 3). The remainder, less than or equal to 30%, will experience procedures of Mild severity (animals on Protocol 1).

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Modelling Paediatric Brain Tumours:

Although in vitro (Petri dish) studies can be used to test how cells become cancerous following gene mutations, this system cannot recapitulate the complex microenvironments existing in developing tissues such as the brain. Because our approach requires the use of specific cancer-susceptible cell types at specific points in brain development, this is currently only possible by using live animals that mirror the complexities and cell populations present in human development. Nevertheless, our judicious use of in vitro cell cultures and our targeted brain injection approach (as opposed to a completely animal-reliant strategy that is focused on producing a small fraction of mice of the correct genetic background from a large number of crossings), greatly reduces the number of mice required. In addition, testing new treatment approaches in the models we will develop has the capacity to highlight different tumour types for specific precision therapies. Together this will dramatically reduce the numbers of animals used for this work and markedly increase the efficiency with which we develop cancer models.

Treating Paediatric Brain Tumours:

Regulatory and research bodies require pre-clinical assessment of potential therapies in animals models prior to their translation into the clinic. Therefore, our translation of optimal new therapies to the children's cancer clinic requires the animal studies proposed here. Nonetheless we will continue to use in vitro drug sensitivity studies, including radiation/chemotherapy combination studies in vitro to optimise the selection of agents and thereby minimise the use of animal models in exploratory studies.

Which non-animal alternatives did you consider for use in this project?

We considered using other approaches such as "mini-brains" called organoids grown in culture dishes, fruit flies or nematode worms for the work proposed here.

Why were they not suitable?

Because "mini-brains", fruit flies and nematode worms lack immune cells, a functional blood brain barrier, the full complement of brain cellular diversity (arguably the most complex organ in our bodies), and the whole body context of a mammal, they cannot generate the data most crucial for our work: the impact of mutations and experimental treatments on how quickly and aggressively brain tumours form and recur.

Our approach requires the use of specific cancer-susceptible cell types at specific points in brain development. This is currently only possible by using live animals that fully recapitulate the complexities and cell populations present in development.

Regulatory and research bodies require preclinical assessment of potential therapies in animal models prior to their translation to the clinic. Therefore, our translation of optimal new therapies to the children's cancer clinic requires the animal studies proposed here. Nonetheless we will continue to use in vitro drug sensitivity studies, including radiation/chemotherapy combination studies in vitro to optimise the selection of agents and thereby minimise the use of animal models in exploratory studies.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken

to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals was determined with advice from a Senior Statistician at the Establishment to ensure that the work will yield reproducible and statistically sound information. Determining the numbers in this way guarantees that the work will not need to be needlessly duplicated in the future. Therefore, animal harm is kept at a minimum. Determining the numbers of animals needed using statistical principles also guarantees that the information and knowledge that is gathered is reliable, accurate and trustworthy. The reliability of this information is very important because it will be used for future, more innovative cancer research.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Our use of intracerebral injections vs an approach relying purely on transgenic breeding to generate brain tumour models, and the use of in vitro cell culture approaches, greatly reduces the number of animals required. Furthermore, our in vivo mouse modelling experiments have been carefully designed with appropriate and stringent statistical rigour in mind, that is aimed at minimising the use of animals while ensuring robust and meaningful endpoints. These animal numbers are selected in collaboration with our highly qualified statistician colleagues and our considerable prior experience with brain tumour mouse models. In addition, we have optimised the use of material from each mouse, often harvesting fresh cells for culture, frozen tumour for genetic studies and fixed material for histology from the same animal. All samples and cells are stored in a frozen state when not in use. This minimises the number of animals required because there is no need to maintain a tumour-bearing colony of living animals at all times. Finally, for every experiment, as part of good practice and prior to any experiments, we will write an experimental protocol which includes clear objectives and methodology including statistical plan and endpoints.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Our use of intracerebral injections vs an approach relying purely on transgenic breeding to generate brain tumour models, and the use of in vitro cell culture approaches, greatly reduces the number of animals required. Furthermore, each of our in vivo mouse model experiments has a careful statistical design that is aimed at minimising the use of animals while ensuring robust and meaningful statistical endpoints. These animal numbers are selected in collaboration with our statistical colleagues and our extensive experience with brain tumour mouse models. In addition, we have optimised the use of material from each mouse, often harvesting fresh cells for culture, frozen tumour for genetic studies and fixed material for histology from the same animal.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Methods : Brain injections of tumour-causing mutations

Mouse models of brain tumours, established using brain injections, will allow us to decipher how tumours form, how they respond to treatments and how the normal cells around them help them grow. Most importantly, mouse models of brain tumours can help us determine which new or experimental treatments show promise in eliminating tumour cells, and how these new treatments are tolerated by the body.

Models: Cross-referencing human tumour cells vs mouse tumour cells

In this project, we want to study how different mutations make brain tumours grow and evolve inside the body.

To do this, we will inject one type of mouse with mouse brain tumour cells and another type of mouse, with a less active immune system, with human brain tumour cells. Comparing human and mouse tumour cells will ultimately allow us to pinpoint exactly what allows these tumours to grow unabated and test treatments that prevents their growth.

Why these methods and models cause the least pain, suffering, distress, or lasting harm to the animals

These methods and models cause the least amount of suffering because the alternative approach to carry out this work would require crossing and maintaining many more genetically altered mice, only a subset of which would go on to develop tumours. In addition, our approach leads to tumour development following brain injections after many months. Prior to the rapid onset of symptoms (at which point mice are killed), the mice develop normally and show no signs of distress or suffering. This combination, the dramatically fewer mice needed to do this work, and the normal development of mice following brain injections, is why this approach causes the least suffering.

Why can’t you use animals that are less sentient?

Less sentient animals such as zebrafish, fruit flies and nematode worms cannot recapitulate the uniquely mammalian biology of the human brain. This is especially relevant to the development of new treatments for brain tumours, which have to be analysed for their effects on not only the tumour, but also on normal brain function and other critical organs in the body. These treatments also have to gain access to the brain through the blood brain barrier. These "extra challenges" facing brain tumour research cannot be studied in less sentient animals because they either do not occur in them or are much more challenging to address. However, they do occur in mice and more easily observed in them. Therefore mice are the most appropriate, least sentient animal models for studying and developing new treatments for brain tumours.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinements will be made routinely over the course of the project and through constant liaison with veterinary and husbandry staff. If needed, amendments to the licence will be applied for, if new procedures are needed to improve animal welfare.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Work in this protocol will be undertaken in accordance with the principles set out in the Guidelines for the welfare and use of animals in cancer research: British Journal of Cancer (2010) 102, 1555-1577.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through constant interaction and engagement with veterinary and animal husbandry staff at the facility including the NVS and the NACWO, annual attendance at NC3R conferences, and through receipt of electronic and printed media from NC3Rs on ongoing improvements to techniques and methodologies. If needed, amendments to the licence will be applied for, if new procedures are needed to improve animal welfare.

Home Office

NON-TECHNICAL SUMMARY

123. Modulating immune responses in the eye to restore tissue health and preserve function

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	pregnant, adult, juvenile, neonate, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To advance understanding of the mechanisms responsible for immune-mediated damage to the eye and to evaluate new treatment to prevent these and restore eye sight.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

For most people, sight is their most important sense and its loss, be it partial or complete, inevitably has profound detrimental consequences. The eye is susceptible to a number of disease conditions that can seriously damage a persons vision. In almost all of these, the underlying cause involves a misdirected inflammatory response that can permanently damage the specialised cells at the back of the eye that are responsible for vision.
The three most important diseases of the eye in the developed world are: 1) Age-related Macular Degeneration (AMD), which cause irreversible blindness due to the progressive loss of specialized cells from the macular region of the retina. 2) Diabetic retinopathy (DR), in which blindness is caused by the abnormal growth of blood vessels, and accumulation of blood and fluid in the macular. 3) Uveitis or intraocular inflammation, is an autoimmune or autoinflammatory condition in which the immune system attacks the eye, leading to damage to the retina.

Collectively these diseases account for over 75% of cases of blindness registrations in the UK. In addition to the personal hardship incurred by sufferers, these diseases impose a heavy financial cost upon the state. The annual direct cost to the NHS of treating these conditions is estimated to exceed £3 billion and the cost to the welfare state of supporting those affected is estimated to be £6 billion/annum. In the UK the prevalence of these diseases is currently rising as a result of an increasingly aged population, the high incidence of obesity (AMD & DR), and the increasing incidence of altered immune responses (Uveitis).
Current treatments for these three diseases is at best only partially effective and relapses occur commonly. Whilst it is clear that in all three, the underlying cause is the infiltration and activation of immune cells into the tissues of the eye, as yet, the factors responsible for initiating the process remain unclear. Consequently there is an urgent need to advance understanding of the molecular signals that initiate these diseases and the cell types which promote the damage. In so doing the proposed study aims to identify biomarkers for these diseases that could be used to enable their early detection and to identify more effective treatment strategies with which to restore the health and function of the eye.
In addition, this study will highlight molecular pathways and cellular interactions that are responsible for inflammation associated with a wide range of diseases, including important autoimmune conditions like osteoarthritis, type 1 diabetes, and multiple sclerosis.
What outputs do you think you will see at the end of this project?

New knowledge: The outlined work aims to advance understanding of the molecular pathways and genetic causes of intraocular inflammation in AMD, Diabetic Retinopathy and Uveitis. As a result of the outlined research, we expect to identify novel targets for therapeutic interventions, the development of biomarkers to detect disease susceptibility as well as disease progression, and identify new approaches that could be used to prevent the development of the disease state.
Publication: The finding of these studies will be presented at national and international meetings including the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO) and published in high-impact peer reviewed journals such as the Journal of Pathology, Nature Medicine, PNAS and Science & Translational Medicine. We are also active in outreach initiatives to patient groups and lay members of the public, where we present and discuss our research findings.

Who or what will benefit from these outputs, and how?

The short-term benefits of the work will be advancing knowledge and understanding of the disease mechanisms that contribute to intraocular inflammation. This will contribute to the scientific development of the different researcher's projects within my group.
In the medium-term (over 2-5 years): the work is expected to benefit researchers working in the fields of ocular disease and inflammation as a result of the dissemination of our research findings through publication. In addition our finding will also be shared with the general public and patients through outreach groups.
In the longer-term (greater than 5 years): The work is expected to benefit medical specialists, working in the field of ophthalmology and patients through the development of more effective treatments for uveitis, diabetic retinopathy and AMD using novel approaches based in part on the understanding of the disease processes developed during this project.

How will you look to maximise the outputs of this work?

Collaborations: The output of the work will be maximised through collaborations with scientists working in other disciplines (engineering, mathematics) who can use data produced in our investigations to develop novel approaches to mathematical modelling and image processing.
Dissemination of research findings: The finding of the studies will be presented at national and international meetings including the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), and published in high-impact peer reviewed journals. We are also actively engaged with patient groups and lay members of the public, where we present and discuss our research findings.

Species and numbers of animals expected to be used

- Mice: 5000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice will be used as the least sentient of the species for which established models of the ocular diseases exist to undertake the proposed studies. In addition, mice are amenable to the genetic alterations needed to undertake the study. Adult mice will be used as the imaging techniques used to monitor the disease are easier to perform at this stage of development.

Typically, what will be done to an animal used in your project?

The purpose of the studies is to determine the role that inflammation within the eye plays in determining the outcome of three important diseases of the eye; namely, uveitis, age related macular degeneration and diabetic retinopathy. In so doing, we aim to identify interventions and treatments that could be used to minimise the associated sight loss.
A significant proportion of the mice used in these studies will be genetically altered, however these alterations are not expected to have any harmful effects. Most of the studies involve the initiation of the disease by either

injecting substances into the eye or damaging the retina by exposing it to a bright light or laser. All of these procedures will be performed under general anesthesia, so the mice do not experience any pain or discomfort and upon recovery from anesthesia behave completely normally.
The ocular disease induced is very mild and is not painful, and whilst the animals may experience a degree of sight loss this does not result in any alterations to their normal behaviour. For a small number of experiments, eye disease associated with diabetes will be initiated by injecting the animals with an inducing agent. Although diabetic mice urinate more than usual, they otherwise continue to behave normally.
Following disease induction, the eyes of mice will be examined (usually twice weekly), under general anesthesia, to monitor the progression of the disease. In addition, small blood samples from the tail may be collected (up to once weekly) to monitor their immune response. Some of the animals will be given treatments aimed at preventing or minimising the disease process, usually involving giving a drug by injection, but in a small number of animals (<2%) by the surgical implantation under general anesthesia of a miniature drug delivery device. At the end of the study the mice will be killed using a humane method.

What are the expected impacts and/or adverse effects for the animals during your project?

The disease models used in these studies are mild and, whilst they may cause some sight loss, they do not evoke pain or result in the animals deviating from normal behaviour. Furthermore, in the mouse, vision is a much less important sense than smell, hearing and whiskering. Most of the procedures are conducted under general anesthesia, as a consequence the animals will experience several anesthetics during the course of the study. The administration of agents used during the study will not cause more than mild transient pain. Some of the mice rendered diabetic may experience mild (<10%) weight loss.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Half of animals will only be used for breeding and will only experience the mild severity category. The remaining animals will be in experimental protocols and are in a moderate severity category.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is difficult to advance understanding of the role that inflammation plays in conditions such as AMD, DR, Uveitis and retinal degeneration because patients do not present themselves until the condition is well developed. Furthermore, it would not be practical or ethical to subject patients to the invasive procedures needed to collect ocular tissue and fluid samples required for analysis or to subject them to untested treatments. It is also not possible to replicate the complexity of the eye or the disease processes that affect it and their associated immune response using either ex vivo, in vitro or in silico models. Consequently, there is no alternative to the use of animals for these studies.

Which non-animal alternatives did you consider for use in this project?

To reduce animal use, an on-going emphasis of our research is directed at developing *in vitro* assays to elucidate *in vivo* observations and test hypotheses. The use of immortalized cell lines and primary cells will be used to facilitate initial investigation into how cellular phenotype and signalling pathways are modulated and to validate appropriate drug targets prior to their use in animal models.
To determine whether a therapeutic treatment is likely to translate into man, we will also perform *ex vivo* studies on surplus tissue left over from human transplants.

Why were they not suitable?

Current *in vitro* models are not able to replicate the complex anatomy of the eye or the pathological and inflammatory process that occur during disease. Nor are they suitable for studying how the disease develops through time, which is a crucial aspect of the chronic eye disease that are the focus of this research.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals needed to generate the genetically altered (GA) mice needed for the study is estimated at 500 animals per year. This figure is based on the number required to maintain the different transgenic lines and supply the GA required for experiments.
Experimental usage (across both disease protocols) is estimated at 500 per year. This number is based upon the scientific objectives of current grant funded projects, and equates to 10 experiments (each with 3 replicates). In estimating group sizes for the studies we have undertaken extensive analysis of data from previous experiments and performed power calculations using statistical packages.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Online tools were consulted but by and large they assume the use of parametric statistics, which are inappropriate for clinical assessment of the models of ocular inflammation and assessment by cell counting. We therefore used statistical packages that allowed us to estimate sample sizes. For models of AMD, we have and will continue to use the Experimental Design Assistant (EDA) from NC3R to design our experiments.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use efficient breeding and can allocate both males and females to specific disease models based on susceptibility to induction. Tissue sharing is encouraged where possible between group members, occurring on an ad hoc basis.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Animal models that replicate three important disease conditions of the eye (uveitis, age related macular degeneration and diabetic retinopathy) will be used. The study is reliant on the use of genetically altered animals, although the alterations used have no adverse effects on the animals. Care has been taken to select animal models that induce only mild disease and are none painful, and for which the associated loss of sight does not impair the animal's ability to perform its normal behaviour. The monitoring and assessment of the disease state will be undertaken using non-invasive imaging methods conducted under general anesthesia and consequently involves only minimal distress for the animal. The delivery of agents or collection of blood samples will be undertaken using well established techniques that causes no more than mild transient pain and no lasting harm.

Why can't you use animals that are less sentient?

Mice are the least sentient of the animal species that have eyes that are anatomically similar to humans and for which established disease models for the three diseases under investigation exist.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We continue to refine our methods for the induction and monitoring of ocular disease e.g. during the course of our previous studies we have advanced our anesthetic procedures to enable imaging to be conducted under gaseous anesthesia thereby avoiding the need to inject mice.
Within the research group, unsupervised algorithm and machine-learning based approaches are being actively explored to allow us to analyse and predict disease progression over time, develop more sensitive measures of treatment efficacy and correlate approaches to better quantify immune-mediated tissue changes.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We participate actively in 3Rs seminars, presenting our approaches and learning from others.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through continuing education including attending and contributing to 3Rs focused seminars and lectures.
Engagement with regional NC3Rs programme manager and Animal Services Unit.

Home Office

NON-TECHNICAL SUMMARY

124. Modulating Radiosensitivity in Tumour and Normal Tissues

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cancer, Tumour microenvironment, Radiosensitisers, Tumour hypoxia, DNA repair

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this project is to improve the efficacy of radiotherapy given to cancer patients without exacerbating the side effects by using novel compounds that enhance the therapeutic response to radiation, named radiosensitisers.
Therefore, this project is mainly focused upon the interactions between radiation therapy and the tumour microenvironment, with an emphasis on the DNA damage response. We are asking whether manipulation of the tumour microenvironment can improve tumour response to radiation, whether alone or in combination with established chemotherapeutic and immunotherapeutic agents.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Although radiotherapy is frequently given to cancer patients with curative intent, there are many situations in which the outcomes remain very poor. For example, patients with locally advanced nonsmall cell lung cancer typically only have about a 15% chance of surviving 5 years despite receiving high dose radiotherapy with concurrent chemotherapy. In addition, radiotherapy can be associated with significant side-effects as a result of damage caused to adjacent healthy tissues which surround the tumour. Therefore, there is an unmet clinical need towards improving outcomes from radiotherapy. The aim of my research is to develop drug treatments which can enhance the effects of radiotherapy on tumour cells without increasing the side-effects of treatment. I anticipate that obtaining this increased understanding of the response of tumours to radiation will lead me being able to translate these pre-clinical findings into clinical studies with the ultimate aim that they will become established therapies for cancer patients.

What outputs do you think you will see at the end of this project?

We will identify the potential of several novel compounds to be clinically effective radiation sensitisers. We will have a greater understanding of the toxicity and efficacy of combining these treatments with radiotherapy. I have previously been able to translate my pre-clinical findings into clinical studies. Based on the *in vitro* data we have obtained with these compounds to date, I am optimistic that these drugs have clear potential to be effective radiation sensitisers.
We will also establish whether these drugs have exerted a synergistic anti-cancer effect, without damaging the surrounding normal tissue, when combined with other treatments such as chemotherapy and immunotherapy. If this work produces promising results, we would anticipate further clinical trials with these novel combinations. We will also aim to understand the mechanisms involved in the tumour response, to potentially open new therapeutic avenues.
The results of the proposed *in vivo* pre-clinical studies will be made available to the scientific community through presentations at National and International Conferences, and publications in peer reviewed journals. Where applicable, the results will also be made available through publicly accessible databases, and the outcomes of this project will be highlighted through the relevant funding bodies.

Who or what will benefit from these outputs, and how?

Although I hope that this work will broaden scientific understanding via conference presentations and publications in peer-reviewed journals, my ultimate aim is to develop effective therapies that are of clinical benefit to cancer patients. The tangible demonstration of this will be whether we can undertake clinical trials with

the most promising compounds that we are studying.

How will you look to maximise the outputs of this work?

We have collaborated with pharma companies as well as the Department of Chemistry in relation to development of the compounds that we will be testing. We have presented our data to scientific and clinical conferences and other meetings. We have also published our data in respected journals such as Nature Communications, Clinical Cancer Research and Science. We will continue to do so in the upcoming work under this licence.
The results of this work will initially be presented to the scientific community through National/International meetings, and appropriate funds have been requested to enable this. The meetings will be carefully selected and will include both smaller specialised conferences, as well as larger international meetings in order to reach both basic research and clinical research audiences. The data will subsequently be published in high-impact journals with relevant open access provision.
We will highlight our findings on relevant departmental websites and where appropriate, our research will be communicated in the form of a press release linked to publication of a journal article. We will utilise social media such as twitter to publicise our findings widely and encourage post-peer review comment on our work.

Species and numbers of animals expected to be used

- Mice: We expect to use up to 4,500 mice over 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse represents a feasible model to test our hypotheses, since multiple reagents, such as antibodies, are readily available, and since the current knowledge on cancer models and responses to therapies is also broadly available. Adult mice, in particular, will be used in this project licence.
With this project, we aim to gain understanding on the tumour response to different therapeutic strategies, including chemotherapy (established substances), radiotherapy, immunotherapy and novel compounds that will be tested under this licence; whether alone or in combination with one or more of the therapeutic strategies named above. The overall goal of the licence is to identify new therapeutic tools that will improve the tumour response to radiotherapy, namely radiosensitisers.
It is therefore, necessary to perform this work in a living organism, given the interplay of the different components that might be responsible for the tumour response, whether within the tumour or belonging to the tumour microenvironment.

Furthermore, we are interested in examining the response of the normal (non-tumoural) tissue to the given therapies, as this would be necessary to validate the suitability of the therapeutic strategy with the aim of bringing these advances to the clinical scenario.

Typically, what will be done to an animal used in your project?

In brief, we will induce tumours in our experimental animals and apply different therapeutic strategies to analyse the tumour response to the treatment. We will also analyse the impact of these therapies in healthy tissue, to validate the suitability of the candidate strategy in a clinical setting.

We will test novel compounds, never administered before to experimental mice. We will first test the safety of such compounds in a dose setting protocol, and determine an appropriate dosing regime in a pharmacokinetics and biodistribution protocol. Typically, experiments of tolerability will extend for a week (single dose, acute toxicity) or for a few weeks (repeated doses, chronic toxicity). A similar scenario is contemplated for experiments of pharmacokinetics.
Our tumour models will be optimised in a tumourigenicity protocol, where tumour cell types, numbers, use of Matrigel and experimental conditions for tumour implantation will be tested and determined based on the scientific needs. The particular involvement of certain genes in tumour formation and tumour response will also be tested under this protocol.
We will use superficial models of cancer, in which tumour cells are subcutaneously implanted in the experimental animal, usually on the flank or the back, under brief recovery anaesthesia. This is the least invasive method to generate tumours to study tumour response to radiation, since only the tumour will receive the radiation, with the rest of the body protected by appropriate shielding, therefore avoiding the involvement of adjacent normal tissue; and because the superficial tumours will not generally interfere with the correct function of internal organs. Tumours will be grown for the minimum time and volume that are needed to deliver the scientific outcome. Tumours will be measured regularly with callipers, a 3D scanner or more advanced imaging modalities (these to be performed under anaesthesia, and on occasions involving the injection of suitable contrast agents). Once the tumour reaches a certain size, different compounds will be administered to the experimental animals. The preferred route would be oral gavage, as considered more refined for the animal. Radiation, in any of the platforms routinely used in our facility will also be applied to the tumours, locally, to assess the tumour response in combination with other therapies.
A particular focus of attention in this licence is the role of the immune system in the tumour response to different therapies. Some experiments designed to fulfil this goal will include implantation of two subcutaneous tumours in the experimental animals. Only one tumour will be treated, with the second tumour being used to test the systemic effect of the treatment given, in particular effects on T-cell responses. This is named the abscopal effect. In another set of experiments to test the role of the immune system in the tumour response, we will aim to test that a successful therapy, manifested by tumour regression, is durable or specific to a cell line. With this aim, we will implant a second or third tumour (once the first tumour has fully regressed) and observe if the tumour growth is impaired. These are the experiments of tumour rechallenge. Finally, to test the role of a particular subset of immune cells, we will be obtaining these from donor mice and introducing them into recipient mice to analyse the tumour response to such intervention, whether in combination with other therapeutic strategies or not. These are the experiments of adoptive transfer. All these experiments represent very interesting scenarios for the clinical set-up and are described in more detail in the section of ‘Action Plan’.
These types of experiments, which are mostly focused on tumour response to the different treatments, are anticipated to extend for eight weeks, approximately. In some cases, experiments of tumour rechallenge upon tumour regression will be performed, and these will typically be completed within six months.
Experiments of normal tissue response are completed within 3-5 days, for irradiation of the abdomen, or within 4 months, for irradiation of the thorax.

What are the expected impacts and/or adverse effects for the animals during your project?

The main adverse effects that the experimental animals would experience under this licence are due to: -

Tumour growth (ulcerations, intramuscular growth, difficulty to eat, pain, body weight loss).

- Administration of substances, some of which might not have been previously tested *in vivo* (toxicity, whether acute or chronic: diarrhoea, dehydration, pain, body weight loss or behavioural changes, choking or cyanosis due to technical error in gavage administrations).

- Radiation, whether applied locally to superficial tumours (skin irritation, ulceration, body weight loss, behavioural changes, pain) or to the normal tissue (gastrointestinal and pulmonary toxicities: abdominal distension, hunched posture, diarrhoea, piloerection; increased or abnormal respiration rate).

- Modified diet (fat enriched): greasy coats, skin irritation due to scratching.

Some interventions will be performed under anaesthesia with the consequent risk of failure to recover. This is particularly relevant for the imaging techniques that some experimental animals will undergo, and which will typically last 45 minutes. Best practice for anaesthesia in small rodents will be applied, and therefore we expect all animals to make a rapid and unremarkable recovery from the anaesthetic.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

It is anticipated that approximately 20% of subject animals will experience moderate severity, with the balance experiencing severity no greater than mild.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Ultimately, we aim to develop radio sensitising treatments which can be combined with radiotherapy to increase the number of patients who can be cured of their cancer without exacerbating side-effects of therapy. For this, we need to gain understanding on the tumour response to different therapeutic strategies, including immuno-, chemo- and radiotherapy with our novel compounds alone or in combination. It is therefore necessary to perform this work in a living organism to understand the interplay between the different components that might be responsible for the tumour response for that particular treatment, whether within the tumour cells or their microenvironment.

Which non-animal alternatives did you consider for use in this project?

As a non-animal alternative, we extensively work with 2D monolayers and 3D spheroids cell cultures. With these models, we determine the most suitable candidate compounds to be further evaluated *in vivo*. Spheroid models recapitulate some features of solid tumours, like the gradients of oxygen and nutrients and, therefore, are a viable alternative to animal models in the context of, metabolism and hypoxia research, for example. In our lab, the use of these models enables us to select a narrower range of candidate compounds for *in vivo* evaluation and considerably reduce the number of experimental animals.

Why were they not suitable?

Any 2D or 3D cell culture models used to analyse the compounds or combination of compounds, have great potential for clinical translation. However, it is necessary to undertake some animal studies with these compounds in order to understand fundamental processes such as bioavailability, distribution, pharmacokinetics and metabolism that underpin cancer within living organisms and which cannot be satisfactorily undertaken *in vitro*. Moreover, we are interested in the role of the tumour microenvironment; such as hypoxia, vasculature or immune response, during cancer treatment, which can almost exclusively be studied in the whole organism.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our estimations are based on our previous knowledge on the variability of the outcomes of interest, for the specific animal models used in our lab.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use a variety of strategies to ensure that the minimum number of mice necessary to achieve the scientific objectives is used in each experiment. We have implemented the NC3Rs experimental design guidelines to design our experiments, for example by ensuring randomization and blinding to avoid bias and increase reproducibility. We use data from pilot experiments in power analysis and statistics-based guidance for future experiments. For example, we determined the effect size from our previous experiments using the mean time to reach a specified tumour size as endpoint. This effect size was then used to calculate the sample size required in our proposed experiment to allow robust comparison between groups. This gave us the overall number of animals included in this project licence.
In order to minimise variables and ensure reproducibility we apply a sturdy methodology in our lab, including the implementation of SOPs for numerous procedures, for example tumour cell culture, preparation and injection, preparation of compounds and vehicles, and their administration to experimental animals, tumour measurement or irradiation procedures, to cite some.

Robust methodology also includes the use of appropriate controls in each experiment, so that conclusions are sound. Each experimental group will differ in only one variable with respect to the correspondent control group, so any differences would be attributable to such variable.
Housing of experimental animals also plays an important role in experimental variability. With this in mind, housing and handling of animals will be to standards that minimise variability. Introduction of microorganisms that could potentially interfere with the results obtained in our experiments will be minimised by the use of individually ventilated cages, the use of appropriate PPE and the implementation of best standard practices for working with immunodeficient animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

New experimental variables to be introduced will generally first be tested in pilot experiments including a reduced number of experimental animals. For example, combination treatments of a novel compound and a well-known compound (such as a common chemotherapeutic agent) will be first tested in a pilot experiment. This will enable us to further investigate the tumour response and determine the schedule/dosing regimes for radiation, chemo- or immunotherapy of a given tumour model with a reduced number of animals. In turn, this will provide the data necessary for the power calculations, to determine the effect size and the minimum number of animals required to achieve a statistically significant result.

During pilot studies, we will obtain data such as tumour growth rate and kinetics, compound levels in blood and other physiological parameters. At the end of the procedure, various tissues and blood will be collected to investigate and optimise protocols for *ex vivo* studies such as immune profiling by FACS or immunofluorescence analyses by microscopy. All these findings will contribute to unveil some of the mechanisms of action of the candidate radiosensitisers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice will be used, as they are the best characterised species for the types of experiments that we propose in this project licence. Furthermore, an array of reagents, including antibodies, are available for this species.
The basic method will be inducing a tumour by injection of tumour cells subcutaneously on the flank or back, as xenograft or allograft tumours. For xenograft studies, immunodeficient mouse strains will typically be used, with naturally occurring or genetically engineered mutations. For allograft models, immunocompetent mouse strains will typically be used in this project. This method of inducing tumours is considered to be more refined, as the animal welfare is not greatly compromised, since it spares normal organs, as compared with other methods (for example, orthotopic or spontaneous tumour models). In some cases, where there is more than one tumour inoculated, the total tumour volume limit for a single tumour will be applied for the combination of both tumours, to minimise suffering due to tumour burden on both sides. We will ensure that the tumour cells we use are optimised for the formation of tumours in the particular strain, for example, tumour cell number, use of Matrigel, tumour characteristics like vasculature or necrosis, with experiments performed under the protocol of tumourigenicity, which will employ a small number of animals.
Tumours will be regularly measured by callipers or a 3D scanner. The 3D scanner provides more accurate measurement and can be done faster by simply restraining the animals and presenting it to the scanner. This will also reduce the time of handling compared to the use of callipers. In some experiments, imaging procedures will be performed to gain more detailed information of tumour growth and physiology. These will be carried out under anaesthesia with appropriate contrast agent when necessary.
Radiation of a subcutaneous tumour is technically much easier in mice because irradiation can easily be delivered without the involvement of adjacent tissues by protecting the rest of the body with appropriate shielding. Radiation is performed under recovery anaesthesia.
To minimise the adverse effects of any novel compounds administered, these will be first tested under dose setting studies to identify the non-toxic doses and routes of administration. Once it’s proven safe, the pharmacokinetics and biodistribution properties will be assessed, and a multiple dosing regime will be tested again in the dose setting protocol. In this way the toxicities should be greatly reduced when these compounds are administered to the mice that we employ in tumour response studies or normal tissue response.

Why can’t you use animals that are less sentient?

Mice are the most commonly used species for the types of studies we are proposing in this project. Mouse models have been extensively used in the development of a wide range of chemotherapies and immunotherapies alone, and in combination with radiotherapy. They are classified as the lowest order species in which we can study tumour growth and adaptive immunity in a way relevant to humans.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For each step in the protocols we have introduced monitoring and controlling measures to minimise the harms for each animal used in this licence. Some of the most significant ones are specified below:
Tumour induction and growth: Tumour induction will be performed under brief recovery anaesthesia, which will facilitate precise positioning of the tumour to minimise the risk of tumour invading the underlying muscle. In the case where a mouse needs two tumour implantations, these will be performed under the same anaesthesia session. Tumour cells will first be tested under the protocol of tumourigenicity to optimise the conditions of tumour formation. Thus, only tumour models that are appropriate will be employed in subsequent experiments performed under other protocols. Tumours will be grown for the minimum volume scientifically needed. Some tumours may develop into an ulcer, which will be monitored for signs of healing for 48 hours and animals will be killed by a Schedule 1 if there is no improvement.
Administration of substances: Before we administer any novel compound that never been administered to experimental mice before, we will prove that the compounds are safe in the dose setting and that the pharmacokinetics and biodistribution properties are also satisfactory. We will use a small number of mice in these experiments. To minimise the adverse effects in experiments performed under the dose setting protocol, we will check the mice immediately after dosing and every 2 hours during the working day, and then daily throughout the treatment period. Mice will be killed by a Schedule 1 method if they lose 15% of body weight and or if any clinical sign would develop (such as changes in behaviour, laboured breathing, hyperactivity, abnormal gait, hunched posture, distended abdomen, diarrhoea). Only substances, administration routes and formulations that are shown not to

cause adverse effects will be used for tumour response experiments and for normal tissue response experiments. Oral gavage will be used whenever possible, as considered more refined than other, more invasive, routes involving injections. For some compounds, it is known that the bioavailability can be increased by increasing the fat content in the diet, which in turn would lower the number of doses of compound to be administered and the amount of compound given at each dose.
Radiation: Radiation will be applied locally to the superficial tumour, under anaesthesia. Damage to non-target tissues is minimised by the use of lead shielding or focussed radiation (including imaging) and precise positioning under anaesthesia. We will explore different irradiation platforms available within facility and apply the most refined whenever possible.
Irradiation of the thorax and abdomen to a maximum dose of 15Gy is not expected to cause any considerable adverse effects if the mice are killed within 4 months of the treatment for lung irradiation and 3-5 days for abdominal irradiation. Normal tissue response will be assessed by imaging and by post-mortem histology.

As a general measure, we will continuously revise our methodology and adapt it according to new policies, technologies and approaches aimed at minimising welfare costs. To achieve this, we will attend the seminars provided by the NC3Rs, keep up to date with NC3Rs recommendations via its website and communicate with local Regional Programme manager for advice to improve the 3Rs in our experiments.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure that experiments are performed under in most refined conditions, we will follow guidelines of best practice based on:

- 'Guidelines for the welfare and use of animals in cancer research' (British Journal of Cancer, 102, 1555-1577, 2010);
- 'Preclinical formulations for discovery and toxicology: physicochemical challenges' (Expert Opin. Drug Metab. Toxicol. 2, 715-731, 2006) for administration of substances;
- 'The Design of Animal Experiments (Reducing the use of animals in research through better experimental design)', Edited by: Michael Festing - University of London, London, UK; Philip Overend - GlaxoSmithKline, UK; Mario Cortina Borja - University College London, UK; Manuel Berdoy - University of Oxford, UK;
- 'Handbook of Laboratory Animal Management and Welfare', Sarah Wolfensohn - BSc, MA, VetMB, Cert LAS, CBiol, FIBiol, Dip ECLAM, MRCVS, Head of Department, Veterinary Services, University of Oxford; and Maggie Lloyd - MA, VetMB, CertLAS, DipHE, MRCVS, Home Office and for guidance from NC3Rs and the University of Oxford 3R's Subcommittee, through their termly newsletters.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

To expand our knowledge on advances in the 3Rs, we will keep up to date with 3Rs newsletters. We will also attend the seminars provided by NC3Rs, keep up to date with NC3Rs recommendations via its website and seek advice to improve the 3Rs in our experiments.

Home Office

NON-TECHNICAL SUMMARY

125. Molecular analysis of key signalling pathways regulating inflammation and innate immunity

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

inflammation, innate immune response, inflammatory signalling pathway, autoimmune/inflammatory disease, infectious disease

Animal types	Life stages
Mice	adult, embryo, pregnant, juvenile, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim is to increase our understanding of the role of specific intracellular signalling pathways (i.e. biochemical interactions within cells) in controlling innate (inborn) immune responses to pathogen infection and their contribution to specific autoimmune/inflammatory diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This work has the potential to identify novel drug targets for the therapy of specific autoimmune/inflammatory and bacterial diseases.

What outputs do you think you will see at the end of this project?

This programme will lead to publications in high impact peer-reviewed journals, of which my laboratory has a good track record. The research will provide important insights into the role of specific signalling pathways in the regulation of immune responses against infections and in pathological immune responses resulting in autoimmunity/chronic inflammation.

REGULATION OF PHAGOSOME MATURATION

Our research is likely to be of broad interest not only to those working on signalling in innate immunity, but also to academic scientists working on infectious diseases and clinical scientists interested in the transfer of basic research on chronic bacterial infections and inflammation to the clinic.

Information gained from our research will be of benefit to academic scientists working across the fields of cell signalling, immunology, cell biology and cancer. The proposed studies on the complex processes involved will also be of considerable interest to researchers working on infectious diseases. In the longer term, this research will inform new methods of targeting the complex in disease.

REGULATION OF PSORIASIS

Genetic studies link our signalling protein of interest to the development of psoriasis. The proposed work will increase our understanding of how this signalling protein induces psoriasis.

The protein is member of a family of related proteins which play important roles in activating other proteins involved in immune responses, autoimmunity and cancer. Some aspects of our work are likely to be of considerable interest to academic researchers investigating how signalling is controlled in immune responses and made dysfunctional in autoimmunity and cancer.

In the short-term, this research will lead to new collaborations with UK-based and international scientists investigating the role of substances involved in the body’s response to other diseases involving these proteins. In the longer term, the research may identify new potential ways of treating psoriasis and be of interest to pharmaceutical companies developing new treatment approaches.

Who or what will benefit from these outputs, and how?

REGULATION OF PHAGOSOME MATURATION

The Programme of Research in this application will determine the role of a specific immune signalling complex in regulating phagosome maturation and immune responses to pathogenic bacteria. This is likely to be of broad interest not only to those working on signalling in innate immunity, but also to academic scientists working on infectious diseases and clinical scientists interested in the transfer of basic research on chronic bacterial infections and inflammation to the clinic.
This research will provide important new information about the functions of the signalling complex in innate immunity and inflammation. This information will be of benefit to academic scientists working across the fields of cell signalling, immunology, cell biology and cancer. The proposed studies on this complex regulation of phagocytosis will also be of considerable interest to researchers working on infectious diseases. In the longer term, this research will inform novel applications of targeting the complex in disease.
REGULATION OF PSORIASIS

Genetic studies link our signalling protein of interest to the development of psoriasis, a chronic autoimmune disease affecting the skin which affects 2-3% of the population. This Project will use biochemical analyses of cultured keratinocytes to determine the molecular mechanism by which this psoriasis-associated protein induces gene expression to promote skin inflammation, identifying key regulators and effectors. The proposed work will increase our understanding of how the psoriasis associated signalling protein induces psoriasis.
The psoriasis-associated protein is member of family of related proteins play important roles in activating NF-κB transcription factors (proteins that switch on the nuclear expression of target genes) in immune responses, autoimmunity and cancer. It is likely that some of the novel regulators identified in this project will also control NF-κB activation by related proteins as well as the psoriasis-associated protein my laboratory researches. Consequently, this project is likely to be of considerable interest to academic researchers investigating how NF-κB signalling is controlled in immune responses and dysregulated in autoimmunity and cancer.
In the short-term, this research will lead to new collaborations with UK-based and international scientists to investigate the role of identified regulators and effectors in other diseases involving these proteins. In the longer term, the research may identify new potential therapeutic targets to modulate its signalling in psoriasis and be of interest to pharmaceutical companies developing new approaches to treat this serious autoimmune disease.

How will you look to maximise the outputs of this work?

Outcomes of our research will be communicated to the academic community by the established routes of peer reviewed publications and presentation at meetings. The principal investigator will present at other academic departments in the UK and abroad, and also attend national/international meetings.
Our work on psoriasis will be communicated to patients via the Psoriasis Association (UK) and the National Psoriasis Foundation (USA) as part of my laboratory's public engagement programme.
Publication of completed studies will be made in journals that adhere to the RC open access policy that ensures immediate access to the published study (Gold) or within no more than six months of publication by depositing in a freely accessible repository (Green).
Our biochemical studies may suggest new ways of controlling the innate immune responses to bacteria or to reduce inflammation in psoriasis.

Species and numbers of animals expected to be used

- Mice: 10,900

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Justification of Species

Genetic manipulation in mice is well established and one of the most powerful means of testing functions and interactions of genes in a live organism. Mice have a relatively short generation time; its blood production system has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. No other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse.

Innate immune signalling in bacterial infection

My laboratory studies the function of two key signalling molecules that are physically associated with one another in a complex. We will use adult mice with fully developed immune systems containing knock-in mutations in which the signalling functions of these proteins are separately blocked by introduction of point mutations. These reagents will allow us to investigate the specific functions of these signalling proteins in innate immune responses to bacteria.

Inflammatory signalling in psoriasis

Recent biochemical and genetic experiments by my laboratory using a human skin cell line have identified a number of novel proteins that control the function of a key signalling protein that is mutated in rare families and induces psoriasis, a chronic autoinflammatory disease of the skin. To establish the physiological significance of these *in vitro* results, we have generated a genetically modified mouse strain which express the mouse equivalent of the psoriasis-associated signalling protein following injection with a specific compound called tamoxifen. Tamoxifen injection of these mice induces skin inflammation 4-5 days later, which has many similarities with psoriatic skin in humans. The functions of the novel regulatory proteins in controlling the signalling function of psoriasis-associated protein will be investigated using our novel mouse model of psoriasis.

Typically, what will be done to an animal used in your project?

Regulation of innate immune responses

My laboratory investigates the function of two signalling proteins that associate with one another in a complex and are critical in innate immune responses. Genetically-modified animals produced to investigate the roles of the signalling proteins we will study are not expected to show any pain, distress, suffering or lasting harm . As such modifications may alter the immune status, animals will be maintained in a barrier environment to minimise the likelihood of compromised health.

Animals bred for our research are not expected (<5%) to experience moderate harm. As it is not possible to fully predict the nature or severity of any potential defect, possible side effects will be closely monitored. We will monitor characteristics of normal mice and collate information on databases to ensure that any mice produced specifically for our work do not show any characteristics not seen in a normal mouse. Animals showing unexpected harmful types will be humanely killed, or in the case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector. Where possible, methods will be modified to reduce suffering (e.g. administration of antibiotics after irradiation, and pain relief pre/post-surgical procedure). In disease modelling experiments, the severity of procedures will be limited by ensuring that animals are killed as soon as overt signs of disease are manifested, with rigorous monitoring for signs of suffering / distress at all times.

A small minority of mice will undergo up to four pre-treatment steps, although the majority will undergo either

none, one or two of these steps in addition to an ‘immunological challenge’. The challenge can consist of substances involved in the immune response, inactivated or live microorganisms or their components which are not expected to cause noticeable clinical symptoms. Substances, including infectious pathogens, may be administered by more than one route.
Monitoring of the immune response may involve withdrawal of blood from a superficial blood vessel, or introduction of substances which can be tracked in the body because they glow under special light.
Levels of stress can affect such assessments and everything will be done to reduce stress levels.

Bacterial infection experiments

Normal and genetically modified mice will be dosed with bacteria either through the mouth, nose, skin or blood system depending on the bacterial species and type of infection investigated. The duration of experiments will be kept to a minimum (maximum 14 days). Disease progression will be assessed by looking for changes relevant to each bacterial species. Mice will be humanely killed. In some instances, we will administer genetically-modified micro-organisms to mice to maximise data and to monitor the course of infection and potential spread to different organs.

Signalling in skin inflammation

Genetically modified mice will be injected with Tamoxifen which induces the production of a protein known to cause psoriasis in humans. Five days after injection, inflammation in ears is clearly visible by laboratory analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Immunological challenge

No adverse effects are expected from these treatments. In some cases administration of pathogen derived products may cause transient inflammation in a proportion (<5%) of genetically-altered animals. These will manifest as partial piloerection (raised fur), hunched posture and subdued behaviour.

Bacterial infection

Airway infection: Most lower airway infections will be short-term (1 week or less). Infection may, in some instances spread throughout the body. Gastrointestinal infection: Most GI infections are mild or with no symptoms and rarely spread in the body. Systemic infection: These infections can cause moderate symptoms quickly and experiments will rarely go beyond a few days in duration. Some animals will occasionally develop mild to moderate symptoms, consisting of reduced activity, piloerection, hunched posture and rapid breathing. During infection animals will be checked at least once a day. It will be rare to continue an experiment beyond 7 days. Endpoints: If animals display one of the signs agreed with the NACWOs and NVS prior to starting the study they will be monitored for 24 hours and advice sought from the NVS/NACWO. If there is no improvement in this time they will be humanely killed. Animals will be weighed at least twice a week if visible symptoms are not expected and weighed daily for infections showing symptoms. For any procedure with no prior data/information, animals will be weighed daily.

Induction of inflammation and/or autoimmunity

The majority of the mutant mouse strains under this programme do not spontaneously develop inflammation and/or autoimmunity. However, the probability of adverse effects is much higher (up to 50%) for mice in which inflammation and/or autoimmunity will be induced deliberately. All methods we use to explore control of the immune system are associated with the following risks and possible adverse effects:
Mice may develop autoimmune / inflammatory diseases (e.g. inflammatory bowel disease), wasting disease or other forms of inflammation (e.g. psoriasis). Humane end points will be taken into account for such conditions.
Wasting will initially be assessed by monitoring changes in growth rate followed by close monitoring of actual loss

of body weight and looking for clinical signs. As soon as 20% of body weight is lost, mice will be humanely killed.

Symptoms of chronic inflammatory bowel disease are diarrhoea, perianal ulceration, intestinal bleeding, occasional rectal prolapse and wasting. If wasting precedes the other symptoms, mice will be humanely killed as soon as 20% of body weight is lost. Alternatively, mice will be humanely killed if symptoms are seen.
Where we investigate the induction of skin inflammation, mice are expected to remain at the mild severity limit for the duration of the experiment. They are expected to display visible scaling of the trunk, ears and tail at day 10-15, by one month scaling is only detected in the ears.
Skin thickening will occur in some animals with only the ears remaining affected at the end of the study.
Mice will be monitored to ensure that any animal showing symptoms exceeding moderate severity for more than 24 hours will be humanely killed.
Whole body irradiation

All mice will be maintained in a barrier environment, to prevent adverse effects on the health of the animal as a result of treatment. The minimum radiation dose will be used. The gut lining may be damaged by radiation which may lead to weight loss and gut inflammation. Microorganisms may enter the circulation, which can result in blood poisoning. To avoid bacterial infections, mice will be given an antibiotic in their drinking water.
Irradiation is expected to result in no more than transient discomfort. However, some mice may develop colitis and such mice will be monitored for signs related to colitis. Mice will be humanely killed if they approach a loss of 20% of their initial bodyweight or have persistent (>24h) diarrhoea or bloating, or prolapsed rectum.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

80% of animals used are expected to exhibit mild clinical signs, with less than 20% displaying moderate severity symptoms.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Studies in cultured immune cells in the laboratory can provide significant information. However, understanding of the role of specific signalling pathways in the regulation of immune responses to pathogen infection and in autoimmune / inflammatory diseases requires the use of living animals.

Which non-animal alternatives did you consider for use in this project?

To investigate how signalling pathways regulate the function of immune cells, my laboratory has initially used

cultured cell lines and primary cells from wild type and mutant mouse strains. These experiments have enabled us to identify critical steps in specific signalling pathways and define mutations that disrupt the function of key component signalling proteins. These studies have allowed us to define a limited set of theories that merit testing in animal models.

Why were they not suitable?

In an immune response, multiple cell types interact with each other and with other cells of the body, and orchestrate a complex series of events culminating in the elimination of the pathogen. Similarly, abnormal activation of the immune system in autoimmune or allergic diseases involves a complex interplay between multiple different cell types. The complex interactions between components of the innate and adaptive immune system with surrounding tissues and organs require whole body model systems as cell culture systems cannot adequately mimic the complexity of tissue interactions that shape immune responses. The mouse is the most appropriate species for these studies because, as a mammal, its immune system is very similar to that of humans, and because of the extensive genetic modification technology that is available for the mouse, and the wealth of pre-existing data and methodology will maximise the net benefit from these studies.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animals have been estimated based on current planned experiments to address key questions. It is expected that each experimental type will need to be carried out three times with group sizes of 5-7 per condition to generate statistically significant data.
To maximise animal usage for one of our projects, male mice will be used as a source of cells for laboratory analyses and animal experiments will be carried out using female mice.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

As much preliminary work as possible was carried out in the laboratory on established cell lines to reduce the number of theories that require testing in animals.
For most of the quantitative experiments, design will be based on ARRIVE guidelines and sample sizes may be set using statistical analysis. Otherwise we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own or from the literature). Pilot experiments will use between 5-8 mice per group, which should be sufficient if a significant result is obtained. The experiment will be repeated to obtain further data if: (a) there are only small differences; in this case it may have to be repeated with larger numbers of mice and/or modifications; (b) does not work; in this case it would be repeated testing other candidate molecules/modulators.
Each experiment (including the pilot ones) will include a small group of animals that are susceptible to infection with the respective pathogen and develop a well-defined course of disease in response to a given dose of

pathogen. This group will serve as an internal control for the experiments involving infections, allowing comparison of results from different experiments (performed at different times, with different doses and/or batches of pathogens etc.). This group will also provide the quality assurance for the virulence of the inocula, which is necessary for comparison of results across experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Mouse experiments will use inbred strains housed under identical conditions to minimise experimental variability. The basic principles of mouse breeding will be adhered to and gestation times, weaning age and litter sizes used to calculate the numbers of mice required to optimise the of the colony.
We will make optimal use of several tissues, fluids and cell types per individual mouse. The highly integrative approach will maximise the information obtained from minimal resources.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

To achieve the objectives in this programme, we propose to use the laboratory mouse as the model organism. The mouse is the best characterised model for these studies, with many features applicable to human infection and autoimmune/inflammatory diseases. Their immune responses are well defined and the technology enabling sophisticated manipulations of the blood production and immune system is highly developed. Genetic techniques in mice are well established; mice have a relatively short generation time; its blood production system has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms.
Animals produced under the breeding protocol are not expected (<5%) to exhibit a moderate outcome. However, we will employ careful monitoring for possible side effects.

Why can’t you use animals that are less sentient?

To our knowledge, no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The severity of procedures will be limited by ensuring that animals are killed as soon as overt signs of the disease can be seen. Most of our procedures are of mild severity, with a minority of animals expected to reach moderate severity.
Where possible, methods will be modified to reduce suffering (e.g. administration of antibiotics after irradiation, and analgesia pre/postsurgical procedure).
Although the pathogens we propose to use in these studies have been studied in mice for a long time and are well understood, new strains of mice and immune modulation regimens will also be tested. This will be first carried out in pilot studies involving a small number of mice.
New strains or batches of pathogens will be tested in pilot studies on small groups of mice which will be carefully monitored for onset of clinical symptoms. This information will be used to adjust the inoculum to a standard level of virulence. It is usual that large batches of inocula are made, tested and frozen in small amounts ready for use at one time, with enough to last around 2-5 years in order that this procedure can be minimised.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

FELASA ‘Working Group on Pain and Distress’, EC ‘Endorsed Severity Assessment’ and NCRI guidelines will be followed and used to determine the earliest endpoint possible on an experiment by experiment basis to allow a valid scientific outcome.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will constantly review our protocols and experimental design to reduce, refine and replace the use of animals.
Frequent meetings are held at our institution on the advances in the 3Rs and their implementation. All staff working under this licence will attend these meetings and frequent advice regarding the well-being of animals under this license will be sought from the vet and animal care staff.
We will follow external sources, including National Centre for Replacement Refinement and Reduction of Animals in Research resources (website https://www.nc3rs.org.uk/) and NCRI guidelines.

Home Office

NON-TECHNICAL SUMMARY

126. Molecular Imaging in Cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

cancer, imaging, therapy, models

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The goal of our work is to create new lung cancer imaging tools for visualising and characterising metabolism in live tumours. By investigating the metabolic profile of these tumours, we aim to determine if new imaging tools

can identify tumours sensitive to new treatments (or combination treatments). We can then subsequently use these tools to guide therapy in individual cancer patients.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

There has been an encouraging increase in the number of cancer therapies over recent years but this has left a challenge. How do we get the right patients on the right drugs quickly? There are several strategies to address this problem but non-invasive imaging is the only technology that can look at the whole tumour as it changes during the course of treatment. Conventional imaging techniques measure the size and location of suspicious lesions and allow doctors to make decisions about patient management but they fail to look at how the tumour is functioning. There is an emerging range of molecular imaging techniques that try to address the problem of assessing tumour function. The most widely applied of these functional assessments is radionuclide imaging, of which the most promising is a technique called positron emission tomography or PET. PET visualises labelled molecules that are injected into the body, these molecules specifically 'home in' to the tumour and give a visual readout of tumour characteristics. Doctors can then use this information to guide treatment decisions, which might include giving a patient a particular therapy or crucially taking them off a therapy that is not working (and may be causing side effects) onto drugs that are most likely to have clinical benefit. This is important in order to get the best outcome for the patient but is also a cost-effective way to administer new cancer drugs. There is not enough data on current imaging technologies to allow them yet to be used to personalise treatment, therefore this project aims to generate that data and take it forward for clinical evaluation. We need better imaging agents that target relevant cancer biology so we also aim to develop these imaging agents and evaluate them as part of this project.

What outputs do you think you will see at the end of this project?

We expect this project to generate data and protocols suitable for publication in peer-reviewed journals which will inform of the clinical application of imaging technologies and an understanding of cancer biology and drug response. Specifically we expect to produce one or two imaging agents that could be taken forward for clinical evaluation by our clinical collaborators.

Who or what will benefit from these outputs, and how?

The purpose of this project is developing better imaging of human cancer and, while this project is not involving humans, we expect that in the short term (1-3 years) we will generate data and products that can be used in clinical trials. We have built extensive relationships with the Respiratory Physicians, Lung Oncologists, Nuclear Medicine Physicians and Radiologists who very keen to further develop the most promising imaging agents developed in this project licence into clinical imaging trials. These trials will aim to provide evidence that specific imaging approaches can be used to guide treatment in clinical trials, predicting whether patients will respond to a particular line of drug treatment or radiotherapy regime.

How will you look to maximise the outputs of this work?

Prior to formal publications the results of this project work will be widely disseminated through conferences, seminars, colloquia and workshops at local, national and international events. We will also share results with collaborators across our various networks and colleague and make resources available to other researchers. Work will also be published in peer-reviewed journals, which are open access.

Species and numbers of animals expected to be used

- Mice: 7500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

To develop tools which are ultimately designed for use in diagnosing human cancer we must use models which most faithfully represent the human condition

Typically, what will be done to an animal used in your project?

Typically mice will be bred in-house to contain specific silenced cancer causing genes. Cancer will be initiated by using of viruses which contain elements that can switch on these silenced cancer genes. These viruses are placed into the respiratory tract of an animal whilst it is anaesthetised to prevent any discomfort and for immobilisation. Lung tumours will then form in the relevant locations in the animal over a period of several months. Tumour growth will be monitored using non-invasive imaging techniques similar to those used in the clinic. At which time we may also use developmental imaging techniques to understand the type of information that these signals represent. Imaging will also be conducted under anaesthesia to reduce movement during scanning which would invalidate the procedure. To maximise the information gained from each animal this imaging may be up to 5 hours in length. We may also repeat this imaging to understand how our imaging signals change during tumour development and to understand the earliest changes which is relevant to early detection of cancer. We may also then provide treatment in the form of drugs or radiotherapy to determine if imaging signal changed after treatment particularly to see if imaging can be used to predict early response to treatment.

Imaging may be repeated during the course of treatment similar to what would be expected in a clinical trial. At the end of the study mice are humanly killed and tissues taken for extensive molecular analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Lung tumour bearing mice can have altered breathing rates as the lung capacity is reduced - this does not typically result in any signs of discomfort. Changes in breathing rate can occur from 2 months onwards and we would normally expect that mice would not be used for longer than 2 months beyond this stage. At the latter stages of disease mice can lose weight and breathing can become more laboured - at this stage mice are typically used for less than 2 weeks. Anaesthesia used in imaging (including when used for imaging mice with lung tumours) can have some short term effects on behaviour including loss of locomotion but these tend to resolve completely within 24-48 hours. Tail vein cannulations cause some damage to the vessels which typically does not cause discomfort and resolves within a period of days.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In most cases we would expect the severity to be mild (70%), moderate (30%) **What will**

happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Cancers are complex and their behaviour is the result of an interplay of several factors including different cells types, poor blood flow and a variable supply of nutrients. Mouse models are the only system that can model this complexity. To evaluate new imaging tools, anticancer agents and radiotherapy we must use these complex model systems to determine their clinical potential.

Which non-animal alternatives did you consider for use in this project?

We considered and are continuing to investigate cells grown in a dish either directly from mouse or human tumours. We also looked at models which contain tumours cells in a way that simulates a living tumour. We investigated the use of computer models which simulate tumour behaviour.

Why were they not suitable?

Experiments on cell grown in a dish can give an important indication of whether an imaging agent will work in a living animal or person but they do not guarantee that it will work. This is because these cell systems cannot reproduce the influence that the circulatory system and other tumour cells have on the delivery of the imaging agent to the tumour and its subsequent clearance. Cell systems also do not reliability predict clinical drug or radiotherapy responses. These are crucial factors that will determine the likely success of any new imaging agents in the clinic.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers are based on experience from the laboratory over the previous 24 months, we also will expand our work due to the start of two new PhD students so this figure has had a 50% increase to account for these personnel.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Animal use will be minimised by using the smallest possible experimental groups in preliminary studies to determine whether a statistically significant effect is obtained with a new imaging agent or intervention (typically six animals in each group; control and test groups). A power analysis may then be used to determine how many

more animals are needed to reach significance. However, if the effect produced by the imaging agent, or imaging protocol, is small then it is likely that the agent will be abandoned at this stage since it is unlikely to be translatable to the clinic. If the effect of an intervention (treatment or radiotherapy) is small (<20%) we are unlikely to be able to measure imaging changes accurately and a new treatment protocol (or new agents) will be considered. Animal use is also minimised by the use of non-invasive imaging techniques in which each animal can act as its own control, for example, before and after treatment. I have also received formal training in experimental design and statistics and include those principles in our experimental planning. In addition, we take advice from our in-house statisticians and use the NC3Rs Experimental Design Assistant or other statistical websites to inform us of our effect size to guarantee our studies are meaningful.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Animal use will also be minimised by inducing tumours through direct tissue injection of agents that result in genetic changes activating cancer causing genes. Such an approach avoids the large breeding programmes and animal use required to produce such genetic changes through animal breeding.

We will also reduce animal use by reducing the experimental unit in our imaging studies down to individual tumours rather than an average of the whole animal. We have demonstrated in previous work that these tumours arise independently in the lung. Individual tumours identified on imaging will be matched to their removed tumour tissue thereby increasing the information that can be gathered for each individual lesion and increasing the power of the study.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Genetically engineered mouse tumour models are becoming increasingly important as systems in which to test new drugs and in which to develop new methods for detecting and predicting the responses of tumours to these treatments. This includes new non-invasive imaging methods, such as those that will be developed in this project. The growth in the use of these models relates to our rapidly growing understanding of the genetic basis of cancer, our ability to genetically manipulate the mouse to produce accurate models of the human disease. The imaging techniques are minimally invasive and thus are not expected to have any significant adverse effects. We will use non-recovery anaesthesia wherever possible but as imaging allow repeated measures in the same mice we also aim to get as much information from each mouse as possible.

Why can’t you use animals that are less sentient?

The development of cancer is a time-dependent process and so tumours do not form in immature mice. Mice are the least sentient species that can be genetically manipulated and provide an accurate model of human cancer development.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We are also refining our methodology to have the least impact on welfare such as improved animal monitoring including temperature and respiration monitoring on multiple mice throughout the imaging procedure. We have

introduced heating during inductions to maintain body temperatures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We adhere to the “Guidelines for the welfare and use of animals in cancer research” Workman et al. (2010) British Journal of Cancer volume 102, pages 1555–1577

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continually review our imaging procedures. Advances in imaging technology are available through international meetings and are published widely in journal and pre-prints. We will engage with these imaging advances as soon as they are sufficiently validated with demonstrated improvement to existing methods. We will take advice from the Named Veterinary Surgeons, Named Training and Competency Officer, Home Office Inspectors and the NC3Rs website. Our technical staff are very proactive in adopting 3Rs advancements such as non-aversion handling and single-use needles and contribute/attend in-house events such as 'culture of care AWERB' and 3Rs workshops.

Home Office

NON-TECHNICAL SUMMARY

127. Molecular mechanisms in eye diseases with vascular complications

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

retina, vision, blood vessels, eye disease, macula

Animal types	Life stages
Mice	juvenile, adult, embryo, neonate, pregnant
Gerbils	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The molecular mechanisms that drive pathology in human eye disease with vascular complications are so far only minimally understood. In this project we aim to gain a better understanding of the mechanisms that might be responsible for these diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The retina critically depends on healthy blood vessels delivering oxygen and nutrients, and removing waste products. This is crucial for normal vision, and complications caused by abnormal blood vessel behaviour in the retina are the main cause of blindness in the Western world. Vision is affected when vessels become leaky or start to proliferate (forming new vessels). This happens in diabetic retinopathy (DR), retinopathy of prematurity (ROP), age related macular degeneration (AMD) and macular telangiectasia type 2 (MacTel). In other instances vessels may become blocked and degenerate, as in diabetic macular ischemia (DMI) or retinal vascular occlusions. In order to develop therapies for these blinding diseases it is imperative to properly understand the cellular and molecular mechanisms that control retinal blood vessel behaviour in health and disease.

What outputs do you think you will see at the end of this project?

The most important insights we anticipate to gain from our work are the elucidation of molecular mechanisms that drive pathology in human eye diseases with vascular complications, which will result in publications and the development of therapeutic approaches to treat those diseases.

Who or what will benefit from these outputs, and how?

Pathology of blood vessels in the retina is the major cause of blindness in the developed world. Because humans so heavily depend on visual function, any visual impairment has major consequences for life quality of the affected individual but also is a significant burden on society. Our efforts to understand the cellular and molecular mechanism that control retinal vascular behaviour in health and in eye diseases, and our aim to develop and test novel therapeutic approaches for such diseases are therefore of major importance, not only for the individual, but also for society as a whole. The groups who will directly or indirectly benefit from this research are diverse. They are patients with blinding eye diseases who need improved therapies, ophthalmologists who need a better understanding of the diseases they are treating and industry who need novel targets to treat those diseases.

How will you look to maximise the outputs of this work?

We are working within the context of a highly collaborative consortium, with very close links to clinical partners and international research groups. Therefore, any progress will be rapidly applied to the benefit of patients. Furthermore, publication of our work will not depend on whether our research hypothesis is confirmed or not, as both outcomes will provide novel insights into the biology of human eye diseases with vascular complications.

Species and numbers of animals expected to be used

- Mice: 1000
- Gerbils: 150

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use mouse pups because their retinal vasculature has a similar stage of development and susceptibility for disease as the retinal vasculature in premature human babies.

Adult gerbils will be used because their retina contains a specialised region for high acuity day light vision as in humans (but not mice). This specialised region in the retina is anatomically and biochemically different from the peripheral retina and shows very different disease susceptibility in humans. We will therefore use gerbils to study macular telangiectasia (MacTel), which in humans only affects the high acuity area of the retina.

Typically, what will be done to an animal used in your project?

Newborn mouse pups will typically be exposed to different oxygen concentrations for up to 10 days. Most of them will also have received one or two injections by the time they are killed humanely when they are around 2-3 weeks old.

The gerbils will typically experience a normal life as our main experimental approach is via the diet modifications, mimicking unhealthy Western diet, which have minimal impact on animal welfare. Most of them will be killed and analysed after 3-24 months of diet exposure.

What are the expected impacts and/or adverse effects for the animals during your project?

In this project notable adverse effects caused by the planned experiments are not anticipated. Gerbils may experience mild distress or pain from injections but otherwise changes to the metabolism and the retina will not be detectable by the animals. Similarly, mice may experience mild distress or pain from injections or sampling, but otherwise changes to oxygen levels and the retina will not be detectable by the animals.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice: 30% sub-threshold, 65% mild, 5% moderate (intraocular injections done under general anaesthesia)

Gerbils: 31% sub-threshold, 62% mild, 7% moderate (intraocular injections done under general anaesthesia)

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Blood vessels are normally perfused with blood and interact with surrounding tissue. These conditions can so far not be recreated in vitro. Therefore it is not possible to study all aspects of vascular behaviour in cell culture experiments.

Which non-animal alternatives did you consider for use in this project?

It is possible to study molecular mechanisms of biological processes in vitro using cultured cells or organoids.
Why were they not suitable?

However, the eye diseases we are studying are complex and the result of interactions between multiple cell types and tissues. Since the details of these interactions are not known yet it is not yet possible to model them in vitro.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Much of this project will use qualitative readouts (that require minimal animal numbers) in multiple iterations in order to optimise and characterise our model systems. It is not possible to predict the success and speed of this approach accurately. Therefore, the animal numbers are based on estimates on how many experimental iterations and hypothesis tests can feasible be achieved within the project duration.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In this project we aim to study phenotypes with large and specific effects that can be measured clearly. This reduces the statistical need for large cohorts of experimental animals. Furthermore, we will use an iterative approach (based on small cohorts) to optimise our model systems. Once this is achieved, power calculations will be used to design proof of principle experiments aimed at therapeutic interventions.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Experiments will be run staggered so that the outcomes of one experiment can be used to optimise the design of the next experiment. Furthermore, we also plan to use global analysis approaches (such as global metabolomics, single cell gene profiling and mass spectrometry imaging) as readouts, which will provide a maximum amount of data per animal and reduce the need for large cohorts (because measuring different genes or metabolites individually needs more sample material and larger cohorts). Lastly, postmortem samples (serum and organs) will be shared with collaborating groups to maximise the insights gained from each animal.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Many of our experimental manipulations target the systemic metabolism in a way that is similar to the challenges seen in humans with unhealthy lifestyles, i.e. they will not cause immediate pain or suffering. In instances where the retina needs to be directly manipulated (e.g. via intraocular injections) or imaged in vivo, no major surgical interventions are required, minimising distress. Furthermore, the majority of experimental readouts will be done under non-recovery anaesthesia and/or postmortem.

Why can't you use animals that are less sentient?

The mouse retina strongly resembles the human peripheral retina, but the human central retina (the macula) is not well modelled in mice because they do not possess a macula. In fact, the only animals that possess a macula are primates and it is often argued that non-human primates are the best animal model to study the human macula. However, we have found evidence that gerbils (but not mice) possess a region in their retina that genetically resembles the human macula and propose here that gerbils are the least sentient animals to model the human macula. Zebrafish, which are less sentient than rodents, may also be used to study retinal biology. However, zebrafish do not have a retinal vasculature and the design and the cellular composition of the fish retina contains crucial differences compared to rodents and humans, making fish unsuitable to address our current research questions.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Recent developments in genetic engineering allow the manipulation of specific genes in a time dependent manner in specific cell populations. This means the systemic effects on the animals by such manipulations can be minimised. We will explore the use of such approaches during this project.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the NC3Rs ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines) and make use of the NC3Rs Experimental Design Assistant (https://www.nc3rs.org.uk/experimental-designassistant-eda).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly check the NC3Rs website (https://www.nc3rs.org.uk/arrive-guidelines) and guides for alternatives such as EURL ECVAM (https://ec.europa.eu/jrc/en/eurl/ecvam), Animal Welfare Information Centre (https://www.nal.usda.gov/awic/alternatives-literature-searching) and
Duke University (https://guides.mclibrary.duke.edu/animalalternatives/databases).

Home Office

NON-TECHNICAL SUMMARY

128.Mouse models of pancreatic cancer and therapy

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cancer, Pancreatic Cancer, Tumour Microenvironment, Fibrosis, Metastasis

Animal types	Life stages
Mice	adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Pancreatic Ductal Adenocarcinoma (PDA) is a dismal disease with late detection, rapid progression and limited treatment options.
The overarching aim of this project is to improve the mechanistic understanding of PDA development. Specifically, we aim to identify mechanisms of PDA pathogenesis with an emphasis on the abundant microenvironment and its role in tumour development, metastasis and therapeutic response in vitro and in vivo.
Specific Objective 1: To define and characterise how the stroma regulates tumour progression, metastasis and resistance to therapy
Specific Objective 2: To identify novel approaches to target the tumour microenvironment
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Pancreatic Ductal Adenocarcinoma (PDA) is the 11th most common cancer (with ~9000 new cases/year in the UK), but is the fourth leading cause of cancer related deaths. The 5-year survival rate is ~9%, which has largely remained unchanged for the last 40 years. PDA is detected late where only 10-15% of all patients have operable disease; however, this is still associated with a 5-year survival rate below 20%. Patients with non-operable disease (~80%) are only offered standard of care chemotherapy, which only marginally increases survival. Although Abraxane and FOLFIRINOX recently have been approved as chemotherapy treatment regimens for PDA, the increased survival is months, not years. PDA is projected to be the 2nd most common cause of cancer-related deaths within a decade and has been denoted as a cancer of unmet need by CRUK.

What outputs do you think you will see at the end of this project?

The primary output of this project is data and novel models – information that advances our mechanistic knowledge of how tumour cells conscribe host stromal cells and how these in turn promote tumour development. Our findings will be made available to the broader public and other scientists through publication in peer-reviewed journals, presentations at scientific conferences and meetings and outreach.
The expected benefits of the work can be summarised as follows:

1) Knowledge of tumour restrictive and promoting signals of the tumour microenvironment

2) Knowledge of the role of individual mutations and associated changes in the tumour microenvironment

3) Knowledge of the role of inflammation and fibrosis in pancreatic cancer

4) Development of more representative models of pancreatic cancer

5) In integrating the knowledge gained from the above, identification of key therapeutic targets for future hypothesis driven therapeutic intervention

6) Test of novel therapeutic agents currently investigated in clinical trials and their potential mechanism of resistance

7) Publication in peer-reviewed journals and presentation at conferences to share the work with the wider scientific community

Who or what will benefit from these outputs, and how?

The main beneficiaries of these outputs will be other scientists, health professionals as well as pharmaceutical companies working on pancreatic cancer. We anticipate that patients won't be able to benefit from this work within the time frame of this project licence, but might benefit in the next 10 years. This might be via better classifying pancreatic cancer and understanding better which patients are likely to respond to current treatments as well as by developing new compounds.

How will you look to maximise the outputs of this work?

Our findings will be made available to other scientists through collaborations, publication in peer reviewed journals and presentations at scientific conferences and meetings. The Establishment has a policy of ensuring that all publications from scientists are available on free access to all.

Species and numbers of animals expected to be used

- Mice: 4500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are more comparable to humans than less sentient model systems (fish, invertebrates) in their pathophysiology and show higher levels of conservation in nucleotide and amino acid sequences. This is important as we intend to use reagents such as small molecule inhibitors and antibodies that have been developed with the aim to treat human pancreatic cancer.
Adult mice are to be used and the development of the disease and pathophysiology closely resemble the human condition.
The pancreas of non-protected species and less sentient species does not sufficiently resemble the human pancreas, so we would be unable to use them for animal models of pancreatic cancer. Embryonic stages would not provide us with a sufficient window to follow tumour development and besides it is not feasible to perform the desired interventions in embryos.

Typically, what will be done to an animal used in your project?

Some mice (bred under other Project Licences) harbour genetic modifications similar to the human disease, which pre-dispose them to the development of pancreatic cancers when exposed to an appropriate agent such as Cre recombinase. In other mice, which are bred specifically to tolerate human tissue, tumours will be implanted under the skin of the mouse for ease of monitoring, or into the pancreas or the bloodstream to study cancer spread (metastasis). Mouse cancer cell lines can be transplanted into mice sharing the same genetic background (so called syngeneic mice) without rejection, which enables the study of the immune system in disease development. Pancreatitis, which is a frequent human disposition, may be induced to accelerate the disease development. Tumour growth is not associated with pain during the period in which we conduct our observations. Tumour growth will be monitored regularly by either use of callipers for superficial tumours, or by imaging methods such as ultrasound for internal tumours. For some procedures that involve surgery under

general anaesthesia, such as implanting human tumour fragments or removing a primary tumour for secondary tumour to grow, we will administer pain killers and monitor the mice closely during recovery.
Some mice may receive either potential novel therapeutic agents, existing clinical agents or placebo administered by a variety of routes, but usually either by mouth, or by injection either under the skin or into the abdomen to study the effects on tumour growth and / or tumour composition. The mice will also have blood samples taken either from the tail vein or by sampling from a heart chamber under anaesthesia (in which case the animal does not regain consciousness before humane termination). Occasionally mice may be administered washing solution and disaggregating enzymes whilst under non-recovery anaesthesia to allow us to undertake analysis of single cells from selected organs.
Mice may be studied for up to 200 days after a period of therapeutic agent treatment for tumour growth. Fast growing tumours will be monitored daily.
Mice will be group housed in ventilated cages which have their environment enhanced with items such as tunnels, houses, nesting material and gnawing blocks.
At the end of any protocol mice will be humanely killed.

What are the expected impacts and/or adverse effects for the animals during your project?

The impact of the gene modifications are not expected to cause any adverse effects per se other than, in the case of the tumorigenic mutations, promoting the propensity to produce tumours. It is possible that the tumour growth might affect normal physiological functions (such as eating, locomotion or breathing) however, mice will be observed daily and any side effect that cannot be managed satisfactorily will be killed humanely.
Injections would only cause very transient pain. Pancreatitis in mice is anticipated to be sub-clinical but will be monitored carefully for signs of pain and discomfort.
After surgical procedures we will monitor mice for signs of pain and administer effective pain relief for as long as it is required.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The vast majority of mice are only expected to experience the mildest clinical symptoms due to tumour growth before they are humanely killed Some mice will experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery with a specialist tube. We will aim to utilise the least stressful route of administration wherever possible.
Mice that undergo surgery will be anaesthetised for the operation and receive pain killer post-operatively until pain subsides. Some mice will also have repeated anaesthesia for the purposes of imaging the internal tumours. Whilst loss of consciousness may be distressing this is not painful.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We will only use animals where there is no suitable alternative. Pancreatic cancer is a very complex disease that involves a number of different cell types e.g. epithelial cells, fibroblasts, immune cells, blood vessels, and as yet

no *in vitro* cell culture system exists that faithfully phenocopies this microenvironment. In addition, pancreatic cancer is characterised by a particularly dense extracellular matrix (ECM). The infiltrating cells and ECM deposition, collectively known as the tumour microenvironment, plays a major role in tumour initiation, progression and metastasis. Moreover, drug efficacy is decreased due to low penetrance and an abundance of anti-apoptotic signals.

Which non-animal alternatives did you consider for use in this project?

My laboratory is committed to establish better *in vitro* models that reflect the complex *in vivo* environment. For example, we have made progress in developing models that recapitulate essential features of the tumour mictoenvirnment. We conduct a great deal of research in these systems already and use these to inform our *in vivo* experiments such that the number of animals used can be limited. Moreover, we are continuously evaluating whether *ex vivo* model systems compliment the *in vitro* co-culture models. We will continue to test and develop these *in vitro* and *ex vivo* systems over the next few years to address how well they model human tumours.

Why were they not suitable?

Whilst enormous progress has been made in the field of cancer research using *in vitro* models, there are several questions that can only be addressed using animal models of disease. For example: investigation of therapeutic response and resistance and interactions with the immune system is most faithfully addressed using pre-clinical *in vivo* models. For these reasons, studies on *in vivo* tumour models need to be performed, in which the benefits are weighted against the likely adverse effects, and humane endpoints utilised.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals to be used is estimated as follows:

- I currently have 3 projects which directly interrogate the interactions between tumour cells and the microenvironment in vivo and one project which aims to optimise and refine models of metastasis. In addition, several projects in my laboratory relies on in vivo models to test specific hypotheses generated from in vitro studies.

- A few studies are anticipated to start and develop under the next 5 years which will require extensive analysis of tumour/stromal cell interdependencies and effect of therapeutic intervention.

- The experience from last 5 years and recent increase in the number of animal models that have been used

in the lab.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experiments are designed using the principles outlined in the experimental design tool on https://eda.nc3rs.org.uk/ and reported following the ARRIVE guidelines as well as the PREPARE guidance on https://norecopa.no/prepare/prepare-checklist
Power analyses will be performed to ensure that we use the minimum number of mice to generate significant results. In cases where power calculations are not feasible, group size will be estimated using the resource equation. Moreover, we involve those with statistical expertise to ensure that we are using optimum groups sizes, and hence minimum number of mice, in our experiments, we use optimal procedures to reduce the number of mice. For each implantation experiment with a new tumour cell line, a small number of animals may be used to determine their tumorigenic potential and the required number of cells necessary to establish tumours so that the smallest number of animals can be used for the experiment itself. Similarly, for implantation experiments involving a new stromal cell line or subsets, a small number of animals may be used to determine their biological impact on tumour growth and the required number of cells necessary to establish an effect so that the smallest number of animals can be used for the experiment itself.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot experiments (2-4 animals) may be conducted to estimate the biological effect of an intervention (such as a drug) when not available.
We use optimal breeding strategies to reduce the number of genetically engineered animals.
We take care to ensure that each experiment is appropriately analysed and that the maximum amount of information is gathered thus reducing the need for experiments to be repeated. For example, at the end of each experiment, data is compared to previous studies by appropriate statistical methods (e.g. Kaplan Meier plots of age at endpoint, Mann Whitney analyses using a one tailed distribution to reduce mouse numbers). Where cell transplantation models are no longer needed, cells and/or tissue will be frozen to avoid unnecessary propagation of the model. Finally, in vivo experiments are preceded by relevant in vitro experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Animals expressing the Pdx1-cre transgene together with oncogenic Kras frequently develop papillomas, however, animals with considerable papilloma burden seem to live quite normally without evidence of distress or discomfort. Animals subjected to pancreas-specific delivery of recombinases (eg Flp or Cre) by virus are not anticipated to experience these issues.
We interact frequently with colleagues that have a vast amount of experience with spontaneous mouse models of pancreatic cancer and have learned to recognize symptoms before animals exhibit any signs of pain (such as: reluctance to move, abnormal posture, vocalization or aggression). We are instating standard operating

procedures for animal care and monitoring where animals will be humanely killed, by Schedule 1 method, if displaying signs of loss of body condition or suffering. Social, environmental and behavioural enrichment will be provided.
None of our protocols exceeds a moderate severity level. Despite the occurrence of metastatic disease, the extent is limited, and animals succumb to primary tumour burden. Moreover, we do not see signs that suggest the animals suffer such that a severe classification would be required. In all cases, signs are monitored very carefully so suffering can be kept within or below moderate limits.

Why can't you use animals that are less sentient?

Less sentient animals do not exhibit a similar stromal reaction and histopathological features as human PDA. Mouse is far more similar to humans than other animals and this is critical both for using reagents like drugs developed for human disease and for translating findings to the clinic. Cancers develop over many weeks to months, so use of terminally anaesthetised animals or immature animals is not practicable.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animal suffering will be minimised by making every effort to keep the tumour models employed at the subclinical levels. Wherever possible, this will be achieved by using non-invasive imaging modalities to monitor tumour growth and the development of metastatic disease. In addition, as detailed in the individual protocols, steps will be taken to minimise the severity of the procedures. As such, while cerulean-induced pancreatitis typically is sub-clinical, repeated injections are needed, which will be alleviated by application of analgesia. Finally, we will ensure that all animals receive the highest standard of care, and preventative medicine (including anaesthesia and analgesia where required) will be used.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Relevant published literature will be used as template for experimental design and decision making (Workman et al., 2010. Guidelines for the welfare and use of animals in cancer research. BJC, 102, 1555-1577).
We will follow guidelines of good practice [Morton et al., Lab Animals, 35(1): 1-41 (2001); Workman P, el al. British Journal of Cancer, 102:1555-77 (2010)] administration of substances will be undertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm.
Guidelines for Body condition score. [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23]
Aging mice will be monitored and managed according to Wilkinson et al (2020) Laboratory Animals: 54(3): 225 – 238.
We will consult the NC3Rs guidelines and monitor refinement where such practices are published (NC3Rs website and elsewhere).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinements with our NACWO, NVS and HO inspector.

NON-TECHNICAL SUMMARY

129. Mouse models of prostate cancer initiation & progression.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

prostate progenitors, castration-resistance, immune system, genetic drivers, cancer

Animal types	Life stages
Mice	adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our aim is to improve the understanding of prostate cancer initiation, progression and disease spread (metastasis), in particular the contribution of genetic alterations occurring in diverse cells of origin. Ultimately, by the use of our mouse models, we aim to develop new treatment strategies which can be translated into the clinic.

A retrospective assessment of these aims will be due by 15 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Prostate cancer (PCa) has become the first leading cause of cancer-related mortality in men in the UK, accounting for 15% of all male cancer deaths. PCa patients exhibit highly variable clinical behaviour; some have indolent disease whilst in others it is lethal.
Localized treatments, which include radical prostatectomy, and radiotherapy, are highly effective for prostate cancer patients. However, 20-30% cases relapse. A main challenge for treating prostate cancer patients is a lack of sufficient patient stratification, which result in standardise treatments to a highly heterogeneous, often multifocal disease. Moreover, currently available immunotherapies have limited effects in PCa patients, highlighting the need to study in depth the prostate local environment for development of adequate therapies. The use of mouse model systems, with a complete immune system will allow us to address those questions and pursue novel therapeutic interventions.

What outputs do you think you will see at the end of this project?

The expected outputs of this project would include:
(i) New knowledge regarding the prostate tumour initiating cells, with particular focus on cell types sustaining more aggressive tumours, leading to the lethal stage. The research will contribute to the prediction of PCa subtypes by cell origin, their associated disease outcome and defined their specific molecular network to be targeted to abrogate tumour progression.

(ii) New knowledge regarding the requirement for several genes or cellular pathways for the function of normal or malignant prostate cells (e.g. inflammatory, stress response pathways), with a particular focus on those selectively required for tumour maintenance, metastasis and therapy resistance. The research findings will be published in academic journals and will be of interest to scientists in the field.

(iii) Identification of specific candidate therapeutic targets in PCa, which would be of particular interest to identify new biomarkers and to develop novel therapies to translate into personalized medicine.

Who or what will benefit from these outputs, and how?

We anticipate that a large part of benefits stated above would be seen within the 5-year duration of the project. The main beneficiaries of these will be other scientists, health professionals as well as pharmaceutical companies working on prostate cancer. We anticipate that patients won't be able to benefit from this work within the time frame of this project licence, but might benefit in the next 10 years. Patient benefit might be via better classifying prostate cancer and understanding better which patients are likely to respond to current treatments, as well as novel immunodulatory combinatorial strategies.

How will you look to maximise the outputs of this work?

Our findings will be made available to other scientists through collaborations, publication in high quality journals and presentations at scientific conferences and meetings. Our Institution has a policy of ensuring that all publications generated are available on free access to all.

Species and numbers of animals expected to be used

- Mice: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Cancer development is dependent not only on the changes occurring within the transformed cells, but also on the interactions of the cells with their microenvironment. Mice are more comparable to humans than less sentient model systems (fish, invertebrates) in pathophysiology and show higher levels of conservation in nucleotide and amino acid sequences. This is important as we intend to use reagents such as small molecule inhibitors and antibodies that have been developed to target human proteins.
While this has elucidated many changes in cancer cells, it provides little information about the local environmental factors influencing early-stage cancer development in life. Also certain hallmarks of cancer, such as spread of cancer and blood vessel formation, are impossible to study in vitro (cell culture). Therefore, mouse models are important for studying the in life aspects of human prostate cancer development. Mouse models have been engineered to develop tissue-specific cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. Moreover, non-protected species and less sentient species do not have prostate, so we would be unable to use them for animal models of prostate cancer involving the injection of prostate cancer cells in the organ where they are found in man. Embryonic stages would not provide us with a sufficient window to follow tumour development and besides it is not feasible to perform the desired interventions in embryos (such as tamoxifen injection in pregnant females). Therefore, adult mice are to be used.

Typically, what will be done to an animal used in your project?

Normal and genetically altered mice will be used to investigate the role of certain specific molecular pathways in the induction, progression and metastatic potential of prostate cancer cells.
The animals will have tumours grown in them initiated through either use of cancer prone genetically modified mice; by the use of inducing agents or by the implantation / injection of existing tumour cells / tumour pieces. For one of the protocols to investigate the spread of cancer we will inject cancer cells into the heart chamber with the aim of delivery to bone sites. However, we do not know the eventual locations until we complete the first study. It is possible that there could be multiple site tumours, but we will monitor the mice very closely to measure the total tumour burden and limit to that which is necessary to achieve the scientific target. To mimic some of the testosterone driven prostate tumour processes, some of the mice will, on occasions be castrated, under general anaesthesia and given post operative pain killers (analgesia). Other surgical procedures requiring general anaesthesia and analgesia are tumour implantation (into specific organs of interest), tumour biopsy (a gold standard method used clinically) and the implantation of small drug delivery systems under the skin.

To monitor the tumour growth, a combination of methods will be used: the use of callipers (for subcutaneous

implanted tumours) and imaging under light general anaesthesia will be performed on a number of occasions to monitor tumour growth. On occasions it will also be necessary to inject, by one of several possible different routes, chemical agents that will allow better imaging of the internal tumours. The majority of animals are not expected to show signs of adverse effects that impact on their general well-being. Very rarely the severity of these signs may be such that the humane end points may be reached (i.e. 20% loss in bodyweight) and the mice culled humanely. The majority of the procedures will result in no more than transient discomfort and no lasting harm.
In order to evaluate the contribution of immune cells in the progression of prostate cancer and associated metastasis, some mice will also have endogenous immune cells destroyed by radiation therapy. These same mice will also be injected with replacement immune cells. Some mice will also have current and potential new therapeutic agents administered to help understand both mechanisms involved in the cancer development but also as a means of assessing the usefulness of new potential treatments for prostate cancer and the subsequent spread. Some mice will have a second challenge of tumour administration to determine the duration of therapy efficacy.
As prostate cancer is a disease of old age, and we will also want to investigate how the cancer spreads and develops in other organs, it will be necessary to continue to follow these mice for up to 28 months.
All the mice will be humanely culled at the end of the experiment.

What are the expected impacts and/or adverse effects for the animals during your project?

The impact of tumourigenic mutations are not expected to cause any adverse effects per se as these in most cases only manifest following administration of inducing agents. Following inhalation of inducing agents mice carrying tumourigenic mutations are expected to have prostate tumours. It is possible that the tumour growth might affect normal physiological functions (such as eating, locomotion or breathing). However, mice will be observed daily and any side effect that cannot be managed satisfactorily will result in humane culling of the animal.

Injections would only cause very transient pain.

After surgical procedures we will monitor mice for signs of pain and administer effective pain relief for as long as it is required.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The vast majority of mice are only expected to experience the mildest clinical symptoms due to tumour growth before they are humanely sacrificed. It is possible that there could be, for a small proportion of the total mice used, multiple site tumours following injection of tumour cells into the heart chamber. Additionally, some mice will experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery with a specialist tube. We will aim to utilise the least stressful route of administration wherever possible.

A minority of mice will undergo surgery and these will be anaesthetised for the operation and receive pain killer post-operatively until pain subsides. Some mice will also have repeated anaesthesia for the purposes of imaging the internal tumours. Whilst loss of consciousness may be distressing this is not painful.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 15 March 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

While valuable studies of human cancer are performed using tumour material and cell lines derived from both mice and human samples, the mechanistic understanding of cancer pathogenesis and its dynamics from tumour initiation to metastasis requires use of living animals (in vivo). In particular, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding host and their behaviour is governed by multiple signals originating from both their immediate neighbours and from distant tissues. Genetically engineered mouse models (GEMM) have been designed to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by in vitro studies or indeed even in different in-vivo models such as zebrafish or insects which remain far less complex than their murine counterparts. . Additionally, inducible mice and tissue-specific Cre recombinases are used to restrict mutations to the biologically appropriate tissue.

Which non-animal alternatives did you consider for use in this project?

Use of animals will be minimised by making use of computer modelling (e.g. prostate cancer patients' data) and in vitro model systems. In particular, we will use a variety of in vitro approaches to investigate how manipulation of nucleic acid based targets alter cell behaviour in cultured cancer cells (prostate) prior to undertaking in vivo studies.
Methods to be utilized include cell biology techniques to measure organoid-growing capacity, cell proliferation, survival, migration, invasion, etc., biochemical and molecular biology techniques such as western blotting, enzymatic assays, proteomics, RT-PCR, etc. to study protein function. In addition, we will use molecular pathology (e.g. immunohistochemistry) to substantiate findings from our in vitro models in human tumour samples.

Why were they not suitable?

The study of cells in culture (in vitro) provides us with clues on the mechanisms of cellular processes in a simple and valuable context, which allows the establishment of hypotheses regarding the function of cells in a living animal. However, these systems do not recapitulate the complex cellular interactions described above.
A retrospective assessment of replacement will be due by 15 March 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

The overall aim will be to generate models whereby a measurable effect e.g. reduction in tumour volume or tumour incidence following manipulation of a gene of interest or treatment with a drug can be determined using the minimal number of animals.
Data available from the literature or from pilot studies are used to perform power analysis to determine an appropriate sample size for the definitive experiment. In general, we will use a sample size capable of detecting a 40% practical difference with 80% power and 95% confidence.
Based on past experience, group sizes of between 10 and 30 animals (dependent on the readout, fewer for transplanted tumours compared to spontaneous tumours in GEMM mice) per experimental group suffice. However, for an experiment to be well controlled and meaningful, we may include more than one experimental group. For instance, in implantation experiments where we deplete a gene in a cell line or prostate subpopulation using shRNA, we will use two independent shRNAs targeting the gene as well as a control shRNA. Moreover, we would typically examine more than one model cell subpopulation/line. Likewise, we may use several doses of a drug, or several different drugs or treatment combinations to test a hypothesis.
Considering power, the number of experimental groups, and the number of genes and drug targets we are interested in, we have then estimated the total number of mice to be used over the licence lifetime.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Use of animals will be minimised by (i) making use of in vitro model systems wherever scientifically justified, (ii) use of in vivo bioimaging to follow disease development and response in real time (rather than culling cohorts of mice at defined time points), (iii) inducing tumour initiation by injecting virus in vivo (iv) the use of pilot experiments to test for the extent of an expected phenotype prior to a full scale confirmatory experiment (thus avoiding full scale experiments that may lack sufficient statistical power), and (v) the cryopreservation in multiple aliquots of prostate cancer samples (which eliminates a requirement for continuous production of cohorts of mice with experimentally initiated prostate cancer).
For our engineered models an efficient breeding strategy will minimise the number of mice used to obtain the desired genotype.
Experiments will be appropriately controlled and mice of the same age, genetic background and source used to reduce the variability of results and to produce highly consistent data. Wherever possible and appropriate, a single group of animals will serve as a control for duplicate experimental group. The proposed experimental designs and methods of analysis of the results will follow statistical guidelines and involve discussion with our bioinformatician scientist to provide sufficiently powered studies, minimizing the number of animals used in each experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource.
We will be conducting and recording our experiments to be able to publish our results following the ARRIVE guidelines [https://www.nc3rs.org.uk/arrive-guidelines] and will use randomisation, blinding etc. where

appropriate so as to minimise biases. Furthermore, additional resources may be used to aid experimental design such as the NC3Rs experimental design assistant tool (https://www.nc3rs.org.uk/experimental-design-assistant-eda).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies will be performed if applicable and, after analysis of the results, group sizes for subsequent experiments will be determined based upon these data. As far as possible, multiple parameters will be evaluated in a single mouse. Live imaging of the same animal at multiple time points also greatly reduces the numbers required.
A retrospective assessment of reduction will be due by 15 March 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to humans. Other less sentient non-mammalian species, such as Danio rerio or Xenopus, which lack an urogenital system that is comparable in complexity and anatomy to that of Homo sapiens have been considered and rejected as models.

The conditions under which the experimental animals are kept and used for the proposed procedures are designed for the least possible disruption of natural behaviour and quality of life. We constantly work to improve husbandry and procedures to minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. As detailed in each protocol, there is provision for the appropriate anaesthetic and analgesic regimes as well as appropriate culling methods following guidelines on administration of substances to mice by Morton et al (2000) and working with cancer models (Workman et al 2010).

Why can’t you use animals that are less sentient?

Only a mammalian prostate cancer model system has the potential to accurately mimic both the anatomy and

complex cell biology, including microenvironmental interactions, of human normal and tumoural prostate. Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human malignancies and many reagents exist for the phenotypic characterisation of mouse cells.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The conditions under which the experimental animals are kept and used for the proposed procedures are designed for the least possible disruption of natural behaviour and quality of life. We constantly work to improve husbandry and procedures to minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. As detailed in each protocol, there is provision for the appropriate anaesthetic and analgesic regimes as well as appropriate culling methods.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Relevant published literature will be used as template for experimental design and decision making (Workman et al., 2010. Guidelines for the welfare and use of animals in cancer research. BJC, 102, 1555-1577).
We will follow guidelines of good practice [Morton et al., Lab Animals, 35(1): 1-41 (2001); Workman P, el al. British Journal of Cancer, 102:1555-77 (2010)] administration of substances will be undertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm.
Guidelines for Body condition score. [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23]

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinements with our NACWO, NVS and HO inspector. I am also a member of the AWERB and I am a regular attendee of our Retrospective Review meeting and the 3Rs Poster session, both of which take place annually.
A retrospective assessment of refinement will be due by 15 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

NON-TECHNICAL SUMMARY

130. Mouse Models to Investigate Neurological Dysfunction

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To characterise highly refined preclinical mouse models to help us understand specific intellectual disability and neurodegenerative disorders in humans.
A retrospective assessment of these aims will be due by 10 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The most common cause of death in England and Wales is neurodegeneration (roughly equal first with ischaemic heart disease in Scotland and slightly less impactful in Northern Ireland but still a major killer). Neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, motor neuron disease including amyotrophic lateral sclerosis, spinal muscular atrophies.

Neurodegenerative disease also makes up aspects of syndromes and a prime example is the occurrence of Alzheimer's disease in Down syndrome: having the neurodevelopmental disorder Down syndrome (the most common known genetic form of intellectual disability) is the single biggest risk factor known for early-onset Alzheimer's disease.

We have almost no treatments for neurodevelopmental cognitive deficits (such as Down syndrome) or for neurodegenerative disorders in humans, let alone cures, although they massively impact our lives. Nor do we understand how a neurodevelopmental disorder like Down syndrome results in a neurodegenerative disorder later in life. We require many different types of models, including cells, human tissues, ex-vivo preparations and animals, to be able to understand these human conditions. We will use mice to show us why and how disorders develop from the early embryo to old age, investigating the interactions between different cell types, in order to ultimately understand disease (for example, Down syndrome, intellectual dysfunction and neurodegeneration) and find treatments.

What outputs do you think you will see at the end of this project?

At the end of our project we aim to have new information about the causes of dysfunction in the central and peripheral nervous system that arise from specific genetic mutations affecting cognition and locomotion, and leading to neurodegeneration. We aim to shed light on the earliest processes, some of which may be suitable targets for therapeutics, or for developing much needed biomarkers of specific diseases.

We will also have new, highly refined, animal models, for therapeutics testing including by genetic manipulation such as the use of new gene-therapy based medicines, or gene editing, for example.
Examples of benefits that we expect from current studies from include:

a) Being able to continue to map specific deficits in memory to a small region of synteny to human chromosome 21 in mouse Down syndrome models. In previous studies we have identified five candidate genes for a specific memory deficit in a mouse Down syndrome model; we expect to be able to start assessing the role of such genes during the course of this licence and thereby deliver potential future therapeutic targets, thus in the long-term, outside of the scope of this licence, helping to ameliorate memory deficits in this disorder.

b) In addition, we are aiming ultimately to identify other genes (possibly up to 3-5) that modulate Alzheimer's disease in Down syndrome. We have yet to identify these genes, but current work is giving important new information which confirms there are genetic targets that can be modulated in Down syndrome-Alzheimer disease, and potentially in Alzheimer disease in the euploid population.

c) Using a combination of mouse models and human immortalised pluripotent stem cells we have been able to determine novel gene transcripts in motor neurone disease. During this project we aim to determine the functional significance of these variants on motor neurone disease pathology.

All studies are aimed at eventually taking us to therapeutics for the disorders modelled, and in all cases we work closely with clinicians and other functional/clinical experts to ensure that knowledge is disseminated and opportunities for therapeutic developments are exploited.
All of this research takes place with regular and frequent interaction with a range of specialists including clinicians who are experts in the disorders we study, and basic scientists including behaviouralists, neuroimagers, cell biologists, molecular biologists and other experts, to help ensure we fully capitalise on the information from our mouse models and relate this back to the human condition.

All of this research will be published and disseminated to non-scientists through the charities that fund our work, and the patient/carer groups, and to scientists/clinicians through publications and presenting work at specialised scientific meetings including both academic and industrial audiences, and lay meetings.

Who or what will benefit from these outputs, and how?

Our research has impact over the short-term and in the long-term, after completion of the project.
In the short-term, the biomedical research community will be the main beneficiaries of our work, in that we will create new resources (mouse models) and disseminate new data on our specific disorders of interest and their underlying mechanisms. The mouse models will help in refining models to address specific questions on the pathology of our diseases of interest. The information on affected cell types, aberrant molecular pathways, aberrant changes detected by neuroimaging, aberrant behaviours, interactions between neurons and other cell types will inform biomedical researchers about early stage disease changes.
In the long-term, the resources and data we produce (both of which will be freely available, long-term, either in biorepositories (mice) or in the literature (data)) will be used by us and by others to help provide the basis for producing therapies for the diseases of interest, and possibly also biomarkers for diagnosis and understanding disease progression.

How will you look to maximise the outputs of this work?

Our ethos is that all individual mouse models should be studied by the widest group of experts possible, so that we maximise our understanding of phenotype. We work on neurological disorders, but to fully understand these disorders, we need experts in heart, skin, etc., to assess our mice holistically. Therefore, we have a wide-ranging set of long-term collaborators from different fields to whom we send samples for analysis, and if we find novel phenotypes we will always go to the experts in that area to ask them to follow up our findings.
All new knowledge is disseminated via publication, meetings, talks, academic and lay. Unsuccessful approaches are equally important and we also publish these in our papers and where possible as individual papers.

As well as conventional scientific press we also have an active social media presence and regular communication with other media (newspapers, radio).

Species and numbers of animals expected to be used

- Mice: 42400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of

the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We work with mice because we can relatively easily manipulate the mouse genome to create animals that exactly mimic the genetics of people who have the disorders we work on. Also, until recently it was very difficult to manipulate the genome of many species, other than mouse, and so there is a wealth of mouse strains that are helpful for biology and which are compatible with the novel mice we make.
We study all life stages, from embryos through to old age, because we are interested in the functioning of the nervous system in different disorders, relevant to neurodevelopment and to later neurodegeneration -- noting that generally we do not know when neurodegeneration starts.
Typically, what will be done to an animal used in your project?

Typically, mice will be used for breeding, to keep our mouse colonies going.
There is no 'typical' regime for all our mouse models, the experiments depend on where we are with understanding the features of the mice we are working with.
For example, we have different protocols to assess the whole, awake animals for locomotion, memory, behaviour:
To assess locomotion in our mouse models, typically we may look at how mice walk, and how far they walk, by giving them free access to a running wheel and then timing how long they are on the wheel, and how far this adds up to running over a particular time period. We might also assess truncal strength by putting a mouse onto a wire hanging above a cage, and timing how long it takes for the mouse to haul itself onto the wire and run along it to the cage edges.
To assess memory, typically we may place a mouse into a box shaped like a capital T - the mouse is placed at the bottom of the T and will explore along the stem up to the T-junction and then turn right or left. We will then take the mouse out of the box, and put it back at the bottom of the T. Because mice are foraging animals, a normal mouse will again run up the stem of the T but then turn in the opposite direction, because it wants to explore. Mice with memory deficits may have forgotten which direction they first went in, and go in the same direction. Thus, we can detect if mutations cause memory deficits.

What are the expected impacts and/or adverse effects for the animals during your project?

Depending on the model of neurodysfunction, we expect to see different features in the mice, most of which will be progressive, most of which are mild or moderate.
For our Down syndrome models, these animals have relatively mild learning and memory deficits, but live to old age - effects are mild but throughout life.

For the motor neuron disease models, these have loss of locomotion which in most cases is mild and throughout life, with our physiological models. Some transgenic models may develop severe symptoms by age four months, but we rarely work with such mice, and then only for a few specific experiments such as to determine the effect of introducing a gene knockout (by crossing in such a locus), as a test for possible therapeutics.
Similarly, for the Alzhiemer's disease models, some are known to have seizures in up to 20% of mice by 6 months of age, but again, these are transgenic models and so overexpress the gene of interest which may lead to the seizures. We have no plans for working with such mice.
For the movement disorders, the phenotypes are also related to locomotion.
We do not have reason to believe that pain is part of any of the phenotypes we study. There may be some weight loss depending on the mutation, but generally this is insufficient to be a welfare issue.
In a small number of strains, locomotion and weight loss will be severely affected and mice may reach humane endpoints within a few months.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

25000 mice on the mild breeding protocol are not expected to suffer any adverse effects and the majority will not have any obvious features that are different from other mice.
We primarily work with models that express the normal or a mutant gene at the same level as occurs naturally – this is unlike transgenic mice for example, in which the gene level is usually many times above normal. These mice are 'physiological models'. Therefore, most the features of our mice are mild and slowly progressing.
The majority of our mice with disease causing GAs are physiologically expressing models with mild features in the heterozygous state (i.e. with one copy of the mutant gene, not two) and therefore most will be bred under the mild protocol. When breeding new GA mice, or generating new crosses (e.g. generating mice with two copies of the gene of interest), mice will be bred on the moderate protocol in the first instance. Up to 25% of the 10000 mice may be show 'moderate' features.
In a minority of cases, GAs may lead to severe phenotypes during breeding ages, due to for example, neurodevelopmental abnormalities; up to 50% of the 1000 mice may exhibit these features under this protocol.
Up to 400 mice undergoing a protocol involving surgery will be non-recovery after terminal anaesthesia.
Protocols for characterising the animals, without surgery, involve up to 6000 mice, where up to 90% of animals may experience 'moderate' features.
Severe characterisation protocols will only involve up to 1000 mice, with less than 50% expected to experience severe features.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 10 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are studying diseases of the nervous system. For one set of disorders that involve cognition, many cell types are involved, and we can neither model behaviour other than with the whole animal, nor do we yet know about all the cell interactions and the effect of development so we have to work with animals.
For another set of mainly neurodegenerative disorders, scientist cannot yet grow the mature neurons that we work on, in dishes, nor can they grow the multiple different cell types together that represent the older human brain, in the body with environmental, hormonal, metabolic and other important influences, including ageing. Therefore, although the mouse lifespan is much shorter than that of humans, nevertheless mice go through an ageing process that mirrors our own, and we can study neurodegenerative disease in mice.

Which non-animal alternatives did you consider for use in this project?

Lower vertebrates and invertebrates are used in the field to understand the interplay between genetics and conserved molecular pathways. However, our goal is to understand disease from a systemic viewpoint within the complexities of the mammalian nervous system. Critically, our most refined humanised models involve large genomic replacements – to understand human mutations in the most physiological way possible – that are not currently possible to engineer in other model organisms with available technology.

Why were they not suitable?

Scientists do not know how to grow the adult neurons we need to investigate, cells in dishes cannot recapitulate the complexity of developing and ageing in a body, and the effects such as hormones and metabolism, and we cannot study behaviour on cells.
A retrospective assessment of replacement will be due by 10 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This is based on a combination of previous throughput and power calculations for individual upcoming experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use the minimum number of animals to give us a statistically meaningful outcome, based on a prior statistical tests such as power calculations. We are careful to use control groups that are age-, sex-, genetic background matched, in order to reduce cohort sizes.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We design breeding plans to work with the minimum number of mice that will give us all the experimental and control genotypes that we need. We undertake small scale pilot studies to determine effect sizes for power calculations. We aim that as much tissue as possible is frozen/fixed and banked within our laboratory for use to use for future studies, and to share with collaborators, and this reduces the need to breed and age mice.
A retrospective assessment of reduction will be due by 10 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse is currently the only mammalian species that we can use to examine the full complement of parameters that are measurable in behavioural and physiological changes, cellular and molecular changes arising from neurodysfunctional disorders, in concert with our ability to tailor the genome of these animals to maximise the information gained from each mouse. We also try hard to develop protocols that detect early changes, such as behavioural changes, prior to major deterioration; such detection can only improve with time and as we learn more about mechanisms including from the very earliest stages. Note that we tend to work with physiological mouse models that have mild phenotypes.
Refinement within our experiments includes processing primary cell lines (e.g. fibroblasts from ear clippings) (and these are immortilised and no further biopsies need to be taken), for multiple future use to ask questions at the cellular level. We also work with human cell lines, such as fibroblasts and induced pluripotent stem cells, to complement, our mouse studies, to validate our findings with the human condition.

Why can't you use animals that are less sentient?

We are studying neurodysfunction in the mammalian nervous system, and while mice are different from humans at all levels, we are sufficiently close in evolution that basic mechanisms are almost always conserved, as are genes -- which is not the case in non-mammals. Therefore mice are the model of choice to study mammalian neurodysfunction, also given the number of different mouse models available to the research community that informs our research.
Other vertebrates may be used to understand some of the biology of the disorders we study, but are not the best at recapitulating pathology such as, for example, behaviour. Similarly, while many aspects of the central nervous system are conserved from humans to invertebrates and may help us understand disruption to these tissues, lower organisms such as fish or flies do not harbour the complexity and intricacies present in mammals, and mammalian models better recapitulate complex pathological changes in disease. For example, invertebrate models often carry far fewer genes than mammals and do not have the paralogues or even orthologues found in the mammalian genome, making modelling of some disorders impossible. Genetic systems in fish may be relevant for some disorders but the manipulation of the fish genome is nowhere near as sophisticated as that for mouse, and cannot currently give us the refined models we work with for human disease, and again, fish may not have the specific genes we wish to investigate. For complex genetic disorders such as Down syndrome (a chromosomal disorder) the technology simply does not exist to model such features in any system other than mouse.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For all tests it is important that mice have no additional stress, therefore mice are handled by experienced operatives only, calmly, and are habituated to testing rooms as well as arenas if possible.
For all handling at all three sites of availability, tunnel handling or cupping are used for all mice, for the benefit of

all animals.

For all tests mice are only housed in modified cages or arenas for the minimum time needed to gather meaningful data.
Mice undergoing phenotyping tests have increased monitoring and are removed from tests if they appear to be suffering from an adverse stress reaction, or other unexpected adverse effects of the phenotyping tests.
Through experience, we have refined methods for handling and caring for a minority of GA mice strains that demonstrate aversion to handling and disturbances. In such strains, we limit phenotyping procedures (do not perform phenotyping tests that elicit an adverse reaction), take extra care when changing cages and performing welfare checks (moving more slowly and quietly), and only change cages when absolutely necessary to reduce disturbances.
Mice that have been given anaesthetics are continuously monitored -- in the case of sedation until they are fully recovered. These mice then have regular extra checks when they are taken back to their home rooms. When general anaesthetics are necessary, the combinations with least adverse effects will be used.

Pipelines are designed with thought given to the overall experience of the mouse and the number of type of tests any one animal will go through.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Routes and volumes for administration of substances are taken from LASA guidelines.
All sites of availability conform to the highest level of quality control on all fronts including husbandry, phenotyping and administrative processes.
Standard operation procedures for most tests have been generated using data and expertise from multiple animal houses and can be found at REDACTED guidelines will be followed at all times.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We receive regular information and updates on the 3Rs from all three sites of availability where we work. We also attend 3Rs events. When we develop 3Rs advances ourselves, we present these at 3Rs events.
A retrospective assessment of refinement will be due by 10 January 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

131. Neural basis of spatial learning and memory

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Hippocampus, Memory, Navigation

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged
Rats	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The hippocampus is a brain structure that supports spatial memory and navigation, in all mammalian species. We aim to understand how hippocampal neurons create a 'map' of space, and how this neural map supports navigation during behaviour. Our specific goals are to understand how hippocampal neurons transform basic sensory information to create a representation of space and how dysfunction of these networks underlies the cognitive changes during aging and neurodegeneration.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our work will deepen our understanding of how the brain represents space (places, directions and distances) and how animals learn to localise themselves in space during development. The study of how hippocampal processing is affected by neurodegenerative diseases may identify early pathological markers that could be used for both diagnostic and therapeutic goals.

What outputs do you think you will see at the end of this project?

The project comprises basic scientific research that will increase our understanding of how the brain supports spatial cognition. The outputs of this project will be new scientific findings, describing how neural activity in the hippocampus (and connected brain regions) supports navigation and spatial memory. The outputs will primarily take the form of publications in peer-reviewed journals, but will also be disseminated at academic conferences and to the lay public via popular science initiatives. Materials, data and methods may, where appropriate, be disseminated via internet download.

Who or what will benefit from these outputs, and how?

In the short timescale, the primary beneficiaries will be other scientists working in the field of learning and memory research. Our research will inform our fundamental understanding of how neural networks for spatial memory and navigation work in healthy adults, during post-natal development, and during aging and neurodegeneration.

In the medium term, the basic science knowledge gained will likely inform broader fields of scientific enquiry, some of which have the potential for clinical translation. For example, understanding how memory networks malfunction during neurodegeneration may inform strategies for developing new pharmaceutical interventions, and understanding how memory networks emerge in development may increase understanding of the clinical symptoms of developmental amnesia patients, and inform the search for therapeutic interventions.

In the long-term, a detailed understanding of the neural-network level mechanisms for learning and memory will be an invaluable aid to designing interventions for memory dysfunction of all types (developmental amnesia, normal aging or neurodegeneration).

How will you look to maximise the outputs of this work?

We will disseminate our research by publishing results in peer reviewed journals. We aim to publish all results,

including those that do not confirm our hypotheses.
We will also present our work to academic peers at scientific conferences (national and international), and engage with the popular science media in order to disseminate our results to the general public.
Raw data and analysis methods will be shared with the scientific community (following peer-reviewed publication), to allow other groups to gain insights from our experiments and reduce replication of work.
In order to broaden the academic reach of our work, we will also engage in collaborations beyond our immediate field. For example, we will collaborate with theoretical neuroscientists, to allow better *in silico* models of how the brain works. We will also engage with clinicians and neuropsychologists working with dementia patients, in order to find how understanding neural networks for memory in rodents can translate into aids for diagnosis or therapy in this clinical population.

Species and numbers of animals expected to be used

- Mice: 1500
- Rats: 1200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project will use rodents, in particular rats and mice. Rodents are good at navigating in familiar environments and remembering what has happened to them there. We already know a lot about the anatomy and physiology of their brains, and in particular of the parts of the brain to be studied in this project. This means that we are well-placed to fill in substantial missing section of our knowledge: how patterns of neural activity create memories (spatial memories in particular), and how the recall of those spatial memories can influence behaviour.
Rats have the specific advantage of being docile and displaying high levels of spatial memory and navigational abilities. Mice, on the other hand, offer unmatched access to genetic tools, allowing us to induce specific gene mutations relevant to diseases such as Alzheimer's disease, as well as using genetic techniques to record from and manipulate functionally/genetically/anatomically defined ensembles of cells.

Typically, what will be done to an animal used in your project?

In the most typical experiment, rodents will undergo a surgical procedure under general anaesthesia, to carry out chronic (long-term) attachment of devices for monitoring neural activity to the skull of the animal. Analgesia will be provided during the surgery and during recovery. Following recovery, the attached devices do not, in themselves, cause any pain or distress to the animal.
Animals will then undergo experiments in which neural activity is monitored simultaneously with behavioural memory testing. The connection of attached devices to systems for amplification and recording of neural signals causes no more than momentary discomfort. Animals will typically be motivated to learn using appetitive (desirable) rewards such as sweet foods, and mild levels of food restriction. Low levels of food are not stressful for the animals, and may also be healthier than a diet based on free availability of food.
Neural recording and behavioural testing experiments typically continue for weeks, or even possibly months.
At the end of the experiment, animals will be humanly euthanized, using an overdose of an anaesthetic agent.

What are the expected impacts and/or adverse effects for the animals during your project?

Some animals may feel pain or discomfort during the recovery from surgery (2-3 days). Post-operative analgesia

will be provided to alleviate this.
Some animals may experience excessive weight loss following food or water restriction. In these cases, animals will immediately be removed from the experiment and provided with freely-accessible food and/or water.

In some experiments, specific brain regions may be lesioned (destroyed), in order to test what role these regions have in learning and memory. In rare cases, lesions may be followed by seizures. In the first instance, these will be controlled by administration of benzodiazepines (following veterinary advice). If seizures cannot be successfully controlled using this method, the animal will be humanely killed.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

30% of animals will experience the severity category 'Mild'.
70% of animals will experience the severity category 'Moderate'.
What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The core aim of this project is to understand how patterns of activity in neural networks create representations of the world (for example, maps of an environment, and an animal's place within it), and how these neural representations then drive behaviour. In order to achieve this, we must study the activity of networks of neurons in animals, as the animals are engaged in the behaviours that we are researching (the expression of spatial memory).

Which non-animal alternatives did you consider for use in this project?

Computational modelling, *in vitro* studies of neuronal activity, research in humans, research in less sentient animals (e.g. insects).

Why were they not suitable?

Computational modelling: we have collaborated (and will continue to do so) with colleagues who devise computational models of hippocampal function and we have used these to design experiments and predict their outcomes, but all existing models are extraordinarily simple in comparison to the complexity of the brain, and cannot substitute for experiments themselves.
In vitro studies: whilst *in vitro* studies can (and have) give information about single neurons or small isolated networks, the only suitable method for studying the normal function of neural circuits underlying complex behaviour is by studying neurons in the intact, behaving animal.
Less sentient species: no homologous structure to the hippocampus has been described in less sentient (non-vertebrate) species.
Humans: implantation of chronic indwelling electrodes in humans is only permissible in a very small number of clinical situations and thus is impractical for most research purposes. There is no noninvasive method of

monitoring the firing patterns of groups of individual neurons in humans. Human neuroimaging techniques (fMRI, PET, MEG) lack either fine temporal resolution (PET and fMRI) or fine spatial resolution (MEG), and anyway deal with comparatively large sections of cortex. They cannot provide information about individual neurons.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of experimental animals have been estimated using a) power analyses, b) experience from previous published studies of effect sizes, and group sizes necessary to test effects, c) group sizes necessary to test effects in our own previously published research.
A pure power analysis approach is not always appropriate for *in vivo* neural recording experiments, as the number of animals required will depend on the success rate of neural recording (numbers of neurons per animal). It is therefore necessary also use estimates based on previous experience of similar experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will seek advice from the University's applied statistics advisors.
We will follow the ARRIVE and PREPARE guidelines and use the NC3Rs Experimental Design Assistant.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies, allowing us to explore whether hypothesized experimental effects may be present, before committing larger number of animals.
Technical developments which enable us to monitor the activities of larger numbers of brain cells in each animal.
Use of computational models that enable us to make highly specific testable predictions about the role of hippocampus and other structures in spatial memory, minimizing the number of experiments required to reach a conclusion.
Most procedures involve long-term experimentation with the same animals, allowing for within animal comparisons, which significantly reduces the number of animals needed to reach statistically significant conclusions.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The model animal used will be rodents (rats and mice).

The major methods used will be:

- Behavioural training. This is necessary to assess cognitive learning and memory capabilities in animals. The large majority of these tests will use only appetitive (rewarding) stimuli, hence the only harm is mild food or water deprivation.

- *In vivo* neural recording. This is necessary in order to be able to draw direct functional links between neural activity and behaviour. Neural recording implants do not cause suffering and distress in themselves, hence the potential for pain and suffering is confined to the post-surgical period (in which analgesia will be provided, see below for details).

Why can't you use animals that are less sentient?

The rodent is the most appropriate animal for the study of the spatial functions of the hippocampus. Laboratory rats display high levels of spatial memory and navigational abilities, and their spatial and non-spatial memory has been tested extensively for over 100 years.
In order to investigate which neural circuits support learning and memory, it is necessary to assess neural function in awake and behaving animals, such that direct inferences can be drawn regarding how neural activity patterns influence behaviour.
Less sentient species, for example invertebrates, do not have brain structures homologous to the hippocampus, and thus are not an appropriate model for learning and memory in mammals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Almost all of our behavioural tests will use appetitive (rewarding) stimuli, the only aversive tests used will involve, for example, bright lights/loud noises or mild air puffs. Animals will be motivated to seek reward using mild food or water deprivation, we will only use the minimum levels of deprivation, for the minimum duration, necessary to achieve uniform consistent behavioural results. Water deprivation will only be used in experiments where food deprivation proves inadequate. On the basis of previous experience, food deprivation will be sufficient in the clear majority of cases (estimated 80-90% of animals used).

Animals are given extensive post-operative care including antibiotics and analgesics where they will be deemed to be necessary. Analgesics are always administered pre-operatively.
Animals are closely monitored throughout the experiments and any signs of problems with implants or other aspects of surgery are immediately dealt with, or, if this is not possible, the animal humanely killed. Similarly, animals are closely observed and monitored during the recording experiments and during interactions with other animals.
Housing cages will be spacious and enriched with rodent toys, chewable materials such as wood, running wheels and a shelter, unless these interfere with the experimental design.
We will use wireless recording systems where possible, in order to eliminate any distress caused to the animal by the recording tether.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Resources hosted on the NC3Rs website, in particular:

- ARRIVE guidelines on experimental design and reporting results.

- 'Procedures with Care': 'Aseptic Technique in Rodent Surgery'.

- Rodent housing and husbandry
- Rat and Mouse Grimace scales

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will use the resources published on the NC3Rs website to ensure that the group undergoes continuous training and professional development with respect to the 3Rs.
We will follow technological advances in the published scientific literature, allowing more efficient recording techniques (yielding more neuronal data per animal), miniaturising recording equipment (leading to a refined animal experience) or allowing recording in more naturalistic settings (for example, wireless recording).

Home Office

NON-TECHNICAL SUMMARY

132. Neural circuit dynamics underlying cognition in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The overall aim of this project is to understand how brain cells communicate and how that supports cognition, the mental operations to obtain knowledge, thought, experience, senses, and complex behaviours. In addition, we will investigate how these functions go wrong in diseases, such as dementia and intellectual disabilities with the aim to find new drug targets or treatment windows for these conditions.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A central question of studying the central nervous system is how complex mental operations in the brain emerge from the constant interactions among nerve cells representing sensory stimuli, motor processes and emotional conditions across various brain regions. Presently the level of knowledge that neuroscientists possess about the underlying biological events that give rise to perception, memories, thoughts, and decisions is very limited. Accordingly, we are still essentially lacking detailed knowledge of root causes for many neurological disorders, including dementia and intellectual disabilities. One possible angle which attracts intense interest is that it might be possible to understand complex mental operations and their defects in disease at the level of a neural circuit, a population of interconnected nerve cells that are collectively activated for a common task.

What outputs do you think you will see at the end of this project?

Our project aims to identify ‘responsible connections’ among nerve cells, whose connection strength is adaptable to mental processes such as learning, memory and decision making in the brain. With such knowledge, scientists will be able to selectively target these connections to enhance their performance in dementia and intellectual disabilities as treatment. Furthermore, nerve pulses among these connections will be detected by medical devices, so that an abnormal pattern of such nerve signals can be used as a ‘marker’ for early diagnosis well before obvious symptoms appear in patients, such as Alzheimer’s disease. In addition, our results may identify a ‘critical time window’ when preventive measures targeting these nerve cell connections can still be effective before they degenerate. These new discoveries will lead to publications, available to the general public.

Who or what will benefit from these outputs, and how?

Our new knowledge towards a detailed understanding of the neural basis of mental processes that generate memories, thoughts and decisions will be valuable for other researchers in basic neuroscience research and raise awareness and curiosity in the general public as to how our brain works.

The new knowledge regarding why, when and how these mental operations collapse in disease will be indispensable for translational scientists, clinicians, and drug companies. In the long run, it will be invaluable for novel strategies and applications in battling against dementia and intellectual disabilities, including AD.

How will you look to maximise the outputs of this work?

We will collaborate or foster potential collaboration with colleagues who share a common interest with us in the physiology of cognition and its dysfunction inside and outside the institution, potentially including industrial partners as well.

For example, we will disseminate all new knowledge internally within the research institute, the university, and

nationally.
We will present our results to British Neuroscience Association meetings and UK DRI Connectome Meetings as well as other informal seminars and symposiums. We will then present our results in international conferences in Europe and the USA to further disseminate research findings internationally. This will include e.g. FENS meetings and SfN meetings.
Finally, we will strive to publish our results in respected journals in the field, such as Neuron, Nature Neuroscience, Nature Medicine, Journal of Neuroscience and eLife.
In these ways, our scientific results and experimental 'know-how' will be shared as early and wide as possible.

Species and numbers of animals expected to be used

- Mice: 6960

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use mice in our project. This is because many cognitive processes happening in humans can be observed or replicated in mice. Furthermore, mouse models of dementia and intellectual disabilities such as Alzheimer's disease are essential for the understanding of the progressive changes in neural circuits responsible for cognitive decline. We will predominantly use adult mice as they are mature and capable of adapting to behavioural training and experimental procedures more effectively.

Typically, what will be done to an animal used in your project?

Some electrophysiological recording and/or optical imaging experiments will be performed in animals under terminal anaesthesia.
In other experiments, animals will first undergo a surgical procedure under general anaesthesia to allow the placement of a head-ring or cannula and then recover before further behavioural training if necessary.
After recovery, behavioural training will be performed If it is required for relevant scientific questions. After behavioural training, animals will be subject to electrophysiological recording (maximal 10 days, once a day) and/or optical imaging sessions (maximal 10 days, twice a day) under fully awake conditions.
Upon the completion of planned experiments mice will be killed by a non-schedule 1 method which is licensed for the purpose of tissue collection, or by a schedule 1 method.

What are the expected impacts and/or adverse effects for the animals during your project?

The maximal level of severity expected in this study is moderate.
For the maintenance of genetically modified mouse lines, we do not generally anticipate adverse effects. If disease mouse models are to be used. Symptomatic animals with negative phenotypes will be maintained only to the humane end-points defined in this licence.
Some of the experiments will be performed on anaesthetised mice. In those the depth of anaesthesia will be carefully monitored. In addition to general anaesthesia, animals will receive local anaesthesia in the surgical sites and analgesia to reduce distress and pain.
In some experiments, animals will first undergo a surgical process under general anaesthesia to allow for example the placement of a head-ring or cannula, and then recover before further behavioural training.
Complications during surgery and recovery are rare, but animals will be closely monitored for any sign of

discomfort, pain or ill health. If complication occurs, they will be dealt with swiftly using standard veterinary procedures.
Some animals will be water or food-restricted in their home cages to motivate them to perform cognitive behavioural tasks for water or food rewards. In such case, animal's weight will be strictly monitored and controlled to a maximal threshold that is approved by the licence, without affecting the animal's general wellbeing.
Specific checkpoints related to specific adverse effects have been detailed in each protocol.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Moderate.
All animals used in this project, except for the maintenance of general genetically modified mice protocol 1 (mild).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The primary objectives of this project are:
1. To understand how real-time neural circuits operate underpinning cognitive demand, such as network oscillations, memory, and decision making.

2. To understand what happens when these mechanisms break down, causing neurological disease such as dementia.

Firstly, it is not possible to manipulate specific neural cell types, the molecular and cellular pathways in healthy humans and those who have diseases e.g. dementia. Therefore we need another method to investigate how these processes impact cognition and its deterioration.
Secondly, cognitive processes cannot be fully recapitulated in vitro, such as cell culture, 2D or 3D organoid and invertebrate model systems. Using an in silico model, such as computational simulation of cognitive processes, it cannot be fully assured without using valid animal models paired with well characterised behavioural tasks.

Finally, transgenic mouse models are powerful tools to render targeted manipulation of cell types, pathways and circuits in vivo. In addition, mouse models of dementia and intellectual disabilities such as Alzheimer’s disease are essential for the understanding of the progressive changes in neural circuits responsible for cognitive decline.
Therefore, using mice in the current research plan is still needed.

Which non-animal alternatives did you consider for use in this project?

We will shift a considerable amount of experimental data generation, such as basic physiological and pharmacological studies to in vitro neural culture, 3D organoid and brain slice preparations.

We will also employ computer-based neural network modelling/analysis.

Why were they not suitable?

Data generated using non-animal alternatives mentioned above are not sufficient to answer the core objectives of this project.
In vitro neural culture, 3D organoid and brain slice preparations can be used to generate hypothesis and to determine cellular and molecular causes or changes in neural circuits associated biology and pathophysiology. But these results can't be directly linked to real-time brain computation processes which are the core question of this project, because these in vitro systems do not generate complex behavioural and (ab)normal memory or executive function.
Silicon computational simulation will provide conceptual predictions of how neural circuits perform in realistic models. But these theoretical leads must be proved or disproved by data generated from a real brain.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Apart from the breeding and maintenance protocols (Protocol 1&2), the numbers of animals to be used are estimated based on the average of estimated sample size (n=15, please see next section for further discussion):
Protocol 1 & 2: We estimate that we need up to 10 breeding cages for each of the 6 transgenic mouse lines, considering changing mating pairs about every half-year over a period of five years. After genotyping, mice with desired genotype and their wildtype littermates will be used in corresponding protocols. Their numbers have been estimated in protocols 3 and 4 (see following). Offspring of mating pairs which are not going to be used in protocols 3 and 4 will be culled.
2 mice X 10 cages X 6 lines X 10 times of changing mating pairs in 5 years = 1200 mice
Additionally, the required number of mating pairs for each line will be monitored 'on-line' and flexible according to our scientific progress. If scientific evidence shows that the active mating for the line is not required, we will reduce the number of mating pairs accordingly.
Protocol 3 & 4: We take the single-cell recording to estimate the likely maximal number of animals we may need because other recording or imaging procedures may be paired with it when using the same mouse. In such a way, we will reduce the total number of animals.
We aim to have N=16 for 6 (an initial estimated number) different recording parameters (such as intrinsic properties, LTP, LTD, spike phase locking, etc) Each parameter requires one recorded cell. (We aim to obtain more parameters from one recorded cell, but this can't be guaranteed.) We will use 1 cell from 1 animal for 2 genotypes for 6 transgenic mouse lines over two different ages. In addition, we may follow 2 different protocols. To be cautious, we also estimate the possibility that one quarter of these experiments (e.g. key findings) may have to be repeated for reproducibility.
16 mice X 6 parameters X 1 cell X 1 mouse X 2 genotypes X 6 lines X 2 ages X 2 protocols X 1.25 repeat = 5760 mice
To sum up, the estimated maximal number of mice for the 5-years period will be:
1200 + 5760 = 6960 mice
About 1100-1400 mice are likely to be used annually according to our protocols to achieve our objectives.

What steps did you take during the experimental design phase to reduce the number of animals being

used in this project?

We are taking the following considerations in the experimental design phase:

1. **Unbiased and randomisation.** First, we will make sure all mice are housed in identical environments with similar husbandry. Second, we will randomly select mice for treatments/measurements in our protocols. Finally, the experimenter will be blinded during experiments as possible as he/she can unless apparent phenotypes prevent such blind design.

2. **Pilot experiments.** For each set of parameter comparison in this project, we will always start with a 'pilot cohort' with a small sample size, such as 6 animals per group. We will be able to improve the quality and efficiency of our overall design with the information obtained from these pilot data.

3. **Power analysis:** After data obtained from pilot experiments, statistics are then used to test whether sufficient animals have been used per group. The same experiment may then be repeated, or not, to test whether our initial design provides sufficient power (e.g. 0.8 or 0.9). Power calculation (https://powerandsamplesize.com/Calculators/) will be used to further test our initial estimation. Besides, the PPL applicant has access to a local full-time bioinformatician/biostatistician for consultation.

4. **Sample size estimation**: In addition to power analysis, the estimated sample size is likely between 10 to 20 to obtain statistically significant results. This estimation is based on previously published work of the PPL applicant, as well as recently published work in well-respected journals by immediate peers in the field (cellular/systems neuroscience).

For instance, in a comparison of 2 means with 2-sided equality, assuming group 'control' mean is 100 and group 'treatment' mean is 70 (expecting a 30% difference), standard deviation as 30, power as 0.8 and α as 0.05, the calculated required sample size is 16.
(http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-Equality)

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

First, lead experiments have been performed by the applicant during his previous scientific training to confirm the technical feasibility of demanding electrophysiological recording (such as in vivo patchclamp recording) as well as quantitative behavioural designs. Besides, the reproductivity for the animals being successful in learning the proposed behavioural tasks within the proposed time window has also been verified.
Second, researchers will receive extensive training for the procedures in the proposed protocols where technical expertise significantly influences the success rate. Therefore, the number of animals to be used will be significantly reduced as experimental 'error testing' using animals will be minimised.
Third, we will maximise the data acquisition from a single animal whenever appropriate during the project. For example, the single-cell recording could be paired with population recording, local field recording or imaging.
Finally, computational simulation and in vitro preparation, such as cell culture, slice culture, acute slices will be used in classical physiology/pharmacology experiments related to the core objectives of this project. This will reduce the overall number of animals to be used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and

methods cause the least pain, suffering, distress, or lasting harm to the animals.

We are using mice because their central nervous system is sufficiently similar to that of humans, which is essential for investigating mechanisms of cognition such as network oscillations (brain waves), memory and decision making We will use wildtype mice and a few transgenic mouse lines, including mouse models of dementia, e.g. Alzheimer's disease and frontotemporal dementia.
We will predominantly perform in vivo single-cell and population electrophysiology recordings and Ca^{2+} imaging in this project. Some experiments will involve training animals to perform cognitively demanding tasks. During behavioural training, we will always keep fine-tuning our protocols and apparatus to minimise stress and increase comfort for the animals.
These methods to our knowledge, are most up-to-date, necessary scientific tools to address objectives set in this project.

Stereotaxic injection

Stereotaxic injection is used to directly introduce pharmacological agents, viral vectors, dyes etc., to targeted regions and/or cell types in the brain. This is more defined than other ways to delivery agents to the brain, such as intravenous or intraperitoneal injection. It is a short procedure performed under general anaesthesia with quick recovery.

In vivo electrophysiology recording

In vivo electrophysiology such as patch-clamp recording or high-density silicon probe recording will be used to record electrical activities (the commutation between nerve cells) in real-time from targeted regions in a computing brain at single-cell or population levels with high spatial (as good as subcellular specificity) and temporal resolution (as good as sub-millisecond specificity). The obtained data are far more informative and precise than traditional sharp electrode or EEG recordings in vivo. To our knowledge, they are the most refined methods in the field to date and will be performed under general anesthetised or awake conditions that animals have been well habituated and trained to minimise stress and discomfort.

In vivo Ca^{2+} imaging

Single or two-photon imaging methods are used to visualise in real-time cellular or neural network activities in targeted brain circuits in a living brain. They can simultaneously provide morphological and activity data with unprecedented detail in vivo. Therefore, they are more refined than other methods to visualise brain activities with high spatial and temporal resolution. These methods will be performed under general anaesthesia or awake conditions that animals have been well habituated and trained to minimise stress and discomfort.

Behavioural training in virtual reality

Virtual reality is used to train head-restrained mice to perform simple cognitive tasks such as spatial navigation and decision making. Virtual reality system provides simulated environments, which can be well controlled with minimised human (experimenter) interference, allowing consistent training/measuring conditions, opportunities to use a wider variety of experimental parameters and more precise control/ measurements of perceptual stimuli/responses of the tested animal. Therefore, behavioural training in virtual reality is more refined than many other traditional behavioural paradigms, and the most appropriate in our project setting.

Why can't you use animals that are less sentient?

The scientific focus of this project is cognition.
Invertebrate models such as fly or zebrafish can be used to study simple responses of neurons to external stimuli and molecular, cellular pathways that cause certain neurological diseases. However, they do not have complex behavioural, memory and cognitive biology and thus it is not possible to study the neural circuitry dynamics that render high-order brain function/dysfunction.

Like humans, mice can integrate sensory and non-sensory information from the outside world as well as their own to command complex behaviours that are vital for their survival, such as foraging, escaping from predators and mating. In addition, mouse models for dementia and intellectual disabilities such as Alzheimer's' disease share many pre-clinical and clinical symptoms including cognitive impairment that

are well characterised in human patients. Furthermore, mice can be readily manipulated genetically allowing target-specific testing of the anatomy and function of neural circuits with high spatial and temporal precision.

Finally, we have planned a significant amount of experiments using mice that will be terminally anaesthetised (see protocol 3) precisely for this consideration.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will use well-established methods and agents for anaesthesia and analgesia in any potentially painful procedures such as surgery. Extra-monitoring/care will be provided to these animals for at least 24 hours after the procedure.
We have made refinement considerations in the following aspects of the project:

1. **Animal husbandry**. Mice will be housed in an enriched environment in their home cages. Small toy items for running, tubes, gnawing sticks for social interaction and safe nesting material will be provided. The disturbance will be kept minimal.

2. **Surgery**. We will carefully select the combination of anaesthetics as well as analgesia. We will calculate the dose for each animal (e.g. via i.p.). Whenever possible, we opt to use the respiratory route, which allows precise control of the depth of anaesthesia and rapid recovery. We also set a post-surgery husbandry scheme (please see details in relevant protocols) to monitor animal's condition to minimise pain and discomfort.

3. **Behavioural training**. Detailed refinement schemes have been provided in the relevant protocols. Briefly, mice will be first made familiar with the experimenter over short daily handling sessions before daily habituation sessions with the apparatus start. We will use a tube to move mice from cage to experimental equipment. Mice will only start to learn tasks when they show no sign of distress.

4. **Behavioural design/equipment**. We will be kept informed with novel designs and new apparatus in the field that can significantly reduce animals' discomfort but provide robust scientifically relevant behavioural readouts. For instance, we are aware of and maybe soon testing the 'Mobile Home Cage' design (https://www.neurotar.com/product/mobile-homecage-mhc-v5/), which may allow mice to navigate more naturally in comparison with virtual reality.

5. **Recoding/imaging sessions**. We have set the maximal number of allowed recording/imaging sessions from a single animal and the maximal length of a single session (Please see details in relevant protocols). Whenever possible, we will reduce the number of repeated sessions and shorten the length of each session.

All these considerations above are aiming for minimising pain, suffering, distress, or lasting harm to the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

First, routes and volumes for the administration of substances are taken from the Laboratory Animal Science Association good practice administration of substances guidelines 1998 (http://www.procedureswithcare.org.uk/lasa_administration.pdf).
Second, we will consult the following guideline specifically for in vivo electrophysiology experiments: Recommendations for the Design and Analysis of In Vivo Electrophysiology Studies *The Journal of Neuroscience*, June 27, 2018 • 38(26):5837–5839

Additionally, we will follow established institutional guidelines for in vivo imaging experiments.

Third, we will follow published local institutional 'Guidelines and Policies for Good Practice' in all procedures involving animals.
Finally, we will comply fully with the following guidelines published by the UK government:
Home Office Guidance:
1. Animal research: technical advice:

Technical advice and guidance for licence holders.
2. Guidance on the Operation of the Animals (Scientific Procedures) Act 1986

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep efficiently up to date with any new paradigms, protocols, technologies that are aiming to refine experimental procedures but at the same time able to maintain feasibility to test our scientific questions.
We will collaborate with colleagues, attend seminars and conferences nationally and internationally to be consistently updated regarding any new procedures which promote animal's welfare in our ongoing project.
At each stage of the project alternative ways to investigate our questions will be considered such as computational models.

Home Office

NON-TECHNICAL SUMMARY

133. Neural mechanisms regulating cardiovascular homeostasis in health, disease and ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Ageing, Neurohumoral regulation, Hypothalamus, Hypertension, Autonomic nervous system

Animal types	Life stages
Rats	adult, aged, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We will expand on the hypothesis that during ageing, the development and/or maintenance of disorders of the cardiovascular system are associated with changes in the expression of a network of genes in the nervous and endocrine systems that form functional networks and that are influenced by lifestyle and nutritional choices, environmental stimuli, age and sex. We will continue to identify key genes and modes of gene regulation in these networks and determine their contribution to the biological system as a whole.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Despite the inevitability of old age, little is known about pathophysiological processes that result in the accompanying homeostatic deterioration. Focusing on two linked aspects of the ageing process, namely osmoregulatory and cardiovascular dysfunction, we will ask how nutritional choices, based around high-salt intake or water deprivation, can act at a genetic level to result in homeostatic imbalance. In order to address the gaps in knowledge revealed by this unmet medical need, we will study the involvement of the nervous system as a master regulator of these functions. In the short-term, the proposed studies will generate important basic knowledge about the way that the nervous system modulates cardiovascular and osmotic homeostasis, and how imbalances lead to disease states. In the
long term, we anticipate that these studies will provide essential information necessary for the development of innovative therapeutic approaches (drugs and gene therapy) for the homeostatic deterioration that accompanies old age.

What outputs do you think you will see at the end of this project?

In the short-term, the proposed studies will generate important basic knowledge about the way that the brain controls the cardiovascular and osmoregulatory systems, and how these mechanisms can go wrong in old age. In the long term, we anticipate that these studies will provide essential information necessary for the development of innovative therapeutic approaches (drugs and gene therapy) for cardiovascular diseases (particularly hypertension). As is our habit, new data in the form of research results will be published in a timely fashion in high-impact, peer-reviewed international journals. By publishing in open access journals, and dissemination at national and international conferences, all data will be freely accessible to the research community. We will share our experiences at 3Rs conferences. All genomic datasets will be deposited in public databases. All novel clones, vectors, animal models, tools and reagents etc. will be offered to the research community upon publication of the primary results subject to costs and availability constraints. Novel viral vectors will greatly benefit the research community. We will modify and update our existing website to enable it to be a forum for communication and dissemination of information about this project.

Who or what will benefit from these outputs, and how?

The proposed research is essentially multidisciplinary in nature and will be of interest to many different research communities, including neuroscientists and physiologists. As we may uncover novel drug targets, the research will also be targeted at pharmacologists. Further, one of the fundamental questions in modern biology is the link between a cell's dynamically modulated transcriptome and its functional phenotype(s). Thus, our exploration of this question in a physiological context will also interest molecular geneticists and anyone engaged in the broad theme of functional genomics. In the medical setting, clinical scientists studying and treating hypertension, cardiovascular and metabolic diseases will find worth in our results. Likewise, our data may influence pharmaceutical companies especially in the medicinal chemistry field. We anticipate that our development of technologies, such as cell-specific viral gene manipulation tools, will be of interest to many scientists as they

seek to refine their work.

How will you look to maximise the outputs of this work?

ACADEMIC
*Project Specific Objective – publication.
We will disseminate our results in relevant high-impact, open-access journals, including opinion pieces where relevant.
Deliverables - a minimum of 10 publications in the most appropriate journals to reach specialists in the field and those with a broader interest
*Project Specific Objective - conferences and seminars.
We will present our work at local and international conferences and at invited seminars.
Deliverables – major local (British Society for Neuroendocrinology (BSN) annual meeting; Society for Endocrinology annual meeting, 3Rs conferences) and international (Experimental Biology, USA) impact.
*Project Specific Objective - collaboration.
I have considerable experience initiating and managing major international collaborations. I have also initiated collaborations with mathematicians with a view to the better exploitation of big transcriptome datasets. We work with leaders in the field to carry out state-of-the-art-work on predictive mathematics, algorithm development, and bioinformatics.

Deliverables – active development of project specific bioinformatics tools and sharing of experience and dissemination with local Bioinformatics Users Group.
*Project Specific Objective - training.
There remains a dearth of scientists with the combination of skills that enables integration of bioinformatics, genomics and molecular genetics with whole animal in vivo physiology, but there is an increasing demand for such approaches within academia and industry. This is being driven by the postgenomic era and the need to determine the functional meaning of genomic data at the whole animal systems level. This project will contribute to the training of young scientists in these areas of high demand.
Deliverables – Intensive bioinformatics training, and continual training and professional development for all participants.
PUBLIC SECTOR
*Project Specific Objective – policy development
We envisage a contribution towards evidence based policy making and influencing public policies and legislation at a local, regional, national and international level. As such we also expect an impact with nutritionists, dieticians and occupational therapists, as well as care-of-the-elderly and geriatrics specialists. It is particularly important that we raise awareness of the importance of proper hydration in the elderly. With the support of the Institutional Policy team, we will develop a focused, targeted and timely engagement strategy that delivers the key messages and policy implications of the findings to the relevant stakeholders. Outputs may include a workshop with relevant policymakers/an interactive tool/case study to demonstrate implications of the research for policy, as well as accessible, targeted policy briefings.
Deliverable – cultivation of links with NHS and public health colleagues.
COMMERCIAL PRIVATE SECTOR
*Project Specific Objective – commercial exploitation
In the event that our results reveal commercial possibilities, these will be eagerly pursued where in the best interests of the research and impact.
Deliverables – regular discussions with our commercialisation team with a view to exploring the possibility of exploiting our findings commercially.
THIRD SECTOR AND THE GENERAL PUBLIC
*Project Specific Objective – public engagement
We view engagement with the public about scientific research an important component of being a scientist.
Deliverable – as the results are rolled out, we will work with the Institution Public Relations team with a view to obtaining press/TV coverage of our work.

Species and numbers of animals expected to be used

- Rats: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In order to understand mammalian cardiovascular homeostasis and its pathologies, it is necessary to study the adult animal, in this case rats, the most tractable model for physiological studies. In some cases, juvenile animals will be examined (before they have developed hypertension). In other cases we will examine the cardiovascular and osmoregulatory morbidities that are prevalent in older rats, as indeed they are in elderly humans.

Typically, what will be done to an animal used in your project?

We want to understand the functions of novel nervous system genes in the control of the cardiovascular system, and how these genes are involved in the development of cardiovascular diseases, particularly hypertension. To do this, we alter the activity of genes or cells in the rat, then monitor the physiological consequences using non-invasive methods where possible. To achieve these ends, surgery is required in order to introduce drugs or genetic tools, or to implant devices into the animal that monitor physiological parameters. Surgery typically lasts for no more than three hours, and recovery is rapid over the following 3 days. We will change the diet of some animals (e.g. high salt) in order to induce high blood pressure and osmotic changes. Experiments can last as little as 48 hours, and usually last for no more than a month. However, as cardiovascular diseases in humans are chronic, it is sometimes necessary to monitor an animal for up to 6 months.

What are the expected impacts and/or adverse effects for the animals during your project?

As a result of surgical procedures, animals will experience short term moderate pain. This will be alleviated as much as possible using analgesic drugs. Some animals will develop high blood pressure. This does not result in obvious effects on activity, health or behaviour.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

About half of the animals will undergo moderate (surgical) procedures. The rest will experience mild (non-surgical) conditions.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are necessary because we propose to investigate the workings of the whole animal, specifically how the nervous system controls the cardiovascular system, and how this goes wrong in high blood pressure. These mechanisms can only be understood within the physiological integrity of the whole organism.

Which non-animal alternatives did you consider for use in this project?

We have considered cultured cell lines. Indeed, for many experiments on basic signalling pathways and transcriptional regulation mechanisms, we use cell lines to establish principles that are then tested *in vivo*.
In addition, the efficacy of all our viral constructs is first thoroughly evaluated in vitro prior to use in vivo. **Why**

were they not suitable?

Cell lines corresponding to adult neurons do not exist and currently available in vitro techniques for the study of the brain, and its interactions with the body, are deficient and are of dubious physiological relevance. Further, these techniques often require the use of use cells and tissues taken from juvenile animals, thus demanding the sacrifice of large numbers of rats.
That said, we are currently exploring the use of organotypic and explant methodologies with encouraging preliminary results.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We estimate that we will study 10 genes in 5 nervous system regions. For each gene we will carry out 3 experiments. Each experiment will involve 20 animals. 10x5x3x20=3000.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use the NC3Rs' Experimental Design Assistant in order to ensure that our plans are critiqued and finely tuned. In all cases, the experimental unit is a single animal. In the past, we pooled animals to give individual experimental units, but thanks to improvements in the sensitivity of techniques, single animals can now be used. Note that technical improvements also enable us reduce animal use by simultaneously extracting DNA, RNA and protein from the same tissue sample.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We comprehensively and non-invasively physiologically assess each animal. These data are compared to molecular data in order to obtain as much useful information as possible. In addition, where possible and valid, we share animal tissue with other collaborating groups in order the maximise data harvesting.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use animal models that enable us to study how the nervous system controls the cardiovascular system, and how these mechanisms go wrong in high blood pressure. The following models are employed:

1. We use a genetic model of hypertension. In the Spontaneously Hypertensive Rat (SHR), high blood pressure develops with age without any surgical or nutritional intervention.

2. We use nutritional models. We alter the availability or content of diet to mimic human conditions that result in cardiovascular changes such as dehydration and high salt consumption. These models are not stressful to the animal.

In these models, we will alter the activity of target genes. In some cases, we will use well-characterised drugs, but in most cases we will use highly specific genetic methods. We use viral vectors to deliver genetic tools to specific brain regions. These genetic tools allow specific genes to be up- or downregulated. We can then monitor the physiological consequences.
We use the most up-to-date non-invasive methods to monitor the physiological consequences of gene manipulation. We use telemetry, which involve implanting a device into the animal. This device either records or transmits real-time data that can then be analysed. At the time of recording, the data acquisition is completely non-invasive in conscious and freely moving animals. This is a great advance over previous methods that used indwelling cannulas.

Why can’t you use animals that are less sentient?

In order to understand mammalian cardiovascular homeostasis and its pathologies, it is necessary to study the adult animal, in this case rats, the most tractable model for physiological studies. We use terminal anaesthesia to harvest tissues, but unfortunately anaesthesia has severe effects on the nervous system that confound and preclude its use in cardiovascular studies. These include changes in gene expression that would affect our transcriptomic and physiological experiments. Indeed, it has recently been demonstrated that anaesthetics uniquely activate hypothalamic neurones (Jiang-Xie LF, Yin L, Zhao S, Prevosto V, Han BX, Dzirasa K, Wang F., 2019, A Common Neuroendocrine Substrate for Diverse General Anesthetics and Sleep. Neuron 102:1053-1065).

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We are fully aware of the requirement to constantly refine our procedures in order to minimise harm. For example, we monitor animals as often as practical in addition to standard post-operative requirements. We will ensure that pain management procedures are the most effective and up-to-date. We have recently implemented a repeated handling protocol to reduce the stress of subsequent procedures.
In this application, we have changed practise we refine our procedures. Examples are as follows:

1. Previously, we used 72 hours of dehydration and 5 days of salt loading. This has been standard practise in the field. However, in this application, we have reduced dehydration to 48 hours and salt loading to 5 days. Our experience over the duration of our previous licence has informed us that the data obtained will be robust and significant without the need for more animals.

2. Instead of subjecting animals to dehydration or salt loading, we are exploring the use of the use of genetic tools. Using viruses, we can target excitatory DREADDS to vasopressin neurones. hence these neurones can be activated by the drug CNO.

3. In the past we have monitored fluid intake and urine output by placing animals in metabolic cages. We will not do this anymore. Instead, we will sample urine from hydroscopic sand.

4. Animals will be group housed after surgery when intake measures are not necessary.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We monitor the comprehensive and invaluable NC3Rs website (https://www.nc3rs.org.uk).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We keep up to date with advances in the 3Rs by attendance at annual meetings of NC3Rs, where we present data and interact and collaborate with fellow participants. We are kept informed of advances by regular communication from the Institutional animal services Unit, and by our local representative on the Animal Affairs Group. We monitor the NC3Rs website to keep abreast of updates (https://www.nc3rs.org.uk).
My Postdocs are members of early career researcher 3Rs group. The purpose of this group is to provide a forum for raising in-house 3Rs-related issues and discuss potential solutions. They will also act as a hub for sharing existing 3Rs approaches from external sources and consider local implementation.

Home Office

NON-TECHNICAL SUMMARY

134. Neuronal mechanisms underlying Rett syndrome

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Rett syndrome, Sleep, Neurons, Learning, Synapses

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this project is to understand how loss of MeCP2, a DNA-binding protein, affects neuronal activity and causes the symptoms of Rett syndrome.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Rett syndrome is the second most common form of intellectual disability in females affecting 1 in 10,000 females. Furthermore, mutations in MeCP2 are found in approximately 1.5% of males with intellectual disability. This number is expected to increase as genome sequencing becomes more widespread. No treatments currently exist for these disorders. Understanding how loss of MeCP2 affects neuronal function may help us identify new therapeutic targets for this disease.

What outputs do you think you will see at the end of this project?

This project will provide us with new information about how loss of MeCP2 leads to Rett syndrome.

Who or what will benefit from these outputs, and how?

The scientific findings from this project will benefit scientists working on MeCP2, Rett syndrome and memory research. In the longer term, beneficiaries may include human patients suffering from Rett syndrome, their families, and the clinicians responsible for their treatment. Thus, this work should have an impact on health and well-being. Improved treatments for MeCP2-related disorders and intellectual disability would be of considerable economic benefit to society in terms of decreased care costs and hours lost to work for families.

How will you look to maximise the outputs of this work?

We will publish our findings in open access scientific journals and present our findings at scientific and family conferences.

Species and numbers of animals expected to be used

- Mice: 1200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The proposed experiments will use adult mice that contain mutations in the MeCP2 protein, which exhibit symptoms that resemble those observed in patients carrying these mutations in MeCP2.

Typically, what will be done to an animal used in your project?

The majority of animals will be used to isolate brain slices for recording neuronal activity. A small number of

animals will have a device implanted in their brain to record neuronal activity during sleep and in the awake state.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of animals will experience no adverse effects. For the small number of animals undergoing surgery, we expect a quick recovery. However, some discomfort may occur pain after surgery. To minimise this discomfort, animals will receive pain relief before and after surgery. To ensure that no significant distress occurs, animals will be monitored daily and a scoring system will be employed, including the Grimace scale, apetite, and behaviour.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of animals (95%) will experience no adverse effects. The remaining 5% may experience some discomfort after surgery.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Mouse models of Rett syndrome exist disorders exist and these mice exhibit symptoms that resemble those observed in patients. Importantly, mice have neuronal network activity that resembles that seen in humans, and therefore serve as important targets for drug discovery. It is only possible to carry out this project in whole animals.

Which non-animal alternatives did you consider for use in this project?

We considered the use of cells derived from human patients.

Why were they not suitable?

It is not yet possible to study neuronal activity and see how it correlates with changes with behaviour using human cells.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our experiments require comparison between mice that lack MeCP2 and normal mice. Due to variation between animals, experiments typically require a minimum of n = 10 mice per experiment. These numbers have been derived from statistical calculations and over ten years of experience performing these experiments.

Much of the data to be gathered in this programme of work will be collected using brain slices. Most of the brain regions that we will study can yield up to six viable slices per animal (three slices containing left and right side of the brain). Therefore, we aim to use slices from one animal on multiple projects simultaneously – thus reducing the number of animals used to provide tissue.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used the proposed techniques for over a decade and generate robust, reproducible data with the minimal number of animals used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have spent considerable effort designing and performing mathematical models to study neuronal activity before experiments are designed. It is only when we have a firm, achievable hypothesis worked out from computational models, that we perform experiments using animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The project will use mice that do not express MeCP2 protein and which exhibit characteristics that resemble those observed in patients with Rett syndrome.

The proposed experiments will examine how loss of MeCP2 changes neuronal activity. Most of the data will be gathered using brain slices prepared from adult mice under terminal anaesthesia. However, the nature of the method implies a great deal of abstraction from the ‘real’ situation in the whole animal. Therefore, it is necessary to validate the results in living mice. We will perform these experiments, wherever possible, under terminal anaesthesia. However, there are times when we will use animals that are anaesthetised. Examples of this include when recording neuronal activity that occurs during sleep.

Why can’t you use animals that are less sentient?

The aims of the proposed work involve examining patterns of neuronal activity that are not present in less sentient species such as drosophila, worms etc. We cannot use mice at a more immature life stage because the neuronal activity in these animals is very different.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Most of the experiments will be performed in terminal anaesthesia and as such mice do not experience any adverse effects of the procedures. Some animals will have electrodes implanted in the brain under general anaesthesia in the animal facility. We will minimise suffering by ensuring the best animal husbandry protocols, daily monitoring of the animal, administration of pre- and post-operative analgesics, and veterinary support is available if required.

The animal units uses a rolling environmental enrichment program ensuring all animals are housed in a varied and stimulating environment. This minimizes stress of the animal and reduces boredom.
Scientists also use the nc3r's initiated tunnel/cupping of animals technique as opposed to tail handling, this reduces stress to the animal and ensures the 3R's are being met.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All members of the laboratory are well trained in the proposed experiments and have personal licenses ensuring that best practises are followed. Lab members continually monitor the literature on these issues are refer to the websites such as nc3rs.org.uk.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Members of the lab routinely attend training forums conducted by experts in the 3Rs. These events involve passing on information on recent advances in the 3Rs and often include presentations by experts in animal welfare include members of the NC3Rs and veterinary surgeons. Attendance at these events has led to changes in our experiments. For example, we stopped picking up mice by tail and instead using tunnels when transferring animals between cages or into equipment. We also routinely monitor developments in the field using websites such as nc3rs.org.uk.

Home Office

NON-TECHNICAL SUMMARY

135.Reward Processing in the brain

Project duration

3 years 2 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

brain, reward, learning, decisions

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The main reason for our work is to advance knowledge about how humans process reward information in the brain. Our work aims to improve our understanding of how rewards are valued based on key characteristics and how this information is used by the brain when making decisions about which reward is best. To do this, we need to know how the brain processes economic (reward) choices that are the most fundamental processes for survival.
We define the word ‘rewards’ scientifically as stimuli, objects, events, situations or activities that are crucial to our individual survival and that of our species. We are interested in how reward mediates learning, approach behaviour (getting closer to an object or stimulus because an animal or human likes it and/or wants it), economic decision making (the process underlying the choice between rewards) and emotions.
The basic way reward and economic decisions are processed in the brain appears to be very similar between humans and non-human primates. This means the non-human primate (NHP) work we do can be used to understand the fundamental brain processes in the healthy and the diseased human brain.
As a result, our work will contribute to our understanding of the processes that drive drug addiction in humans,

A retrospective assessment of these aims will be due by 30 March 2024

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

By understanding the brain signals underlying the reward and economic decision processes will enable us to understand the basic building blocks of reward directed behaviour and explain why such decisions may go right or wrong.
It is now thought that cells called neurones (nerve cells) in the brain detect rewards but not how such 'reward signals' detected by these neurones lead to the choices we make. There are good learning and decision theories available, and we will use them to formulate and answer our questions. For example, how does the brain detect fatty and sugary food and liquid rewards that lead to obesity that is so harmful to so many people? Other examples of our research will look at how uncertain rewards are processed and how this may lead to irrational choices that are harmful to humans.
Knowledge of normal processes is fundamental if we are to understand what goes wrong in reward disorders such as obesity, risk taking and drug addiction. Any, even minor, advances are likely to help the search for treatments that could save thousands of lives.
In addition, because in our daily life we receive many different rewards, we also need to understand how the brain interprets the rewards other humans are getting and how this affects the way we behave. To understand why we misjudge rewards in friendly and unfriendly situations may help us understand how to reduce social conflicts. The knowledge gained from these experiments should help us to understand the abnormal processes in reward addiction, obesity, gambling, attention deficit disorder and other disorders, and provide further steps to improve human health and welfare.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Non-Human Primate, 7-9 animals (transferred from current licence).

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Our NHPs live in rooms that house 2 to 4 animals. This is considered an appropriate number to ensure their welfare. Unfortunately, some animals will fight as they get older and try to establish hierarchies. This can result in injuries and the need to separately house animals for their safety. A separately housed animal will always be able to see, hear and communicate with the other animals living in the same room. If socially compatible, separately housed NHPs will have periods of time during which they are allowed to have direct contact with other NHPs; these contacts will enable them to groom each other and to conduct other social activities; however, these contacts this will be closely supervised.
It takes us several months to train our animals to enter the laboratory from their living area and to perform specific tasks. These tasks include making choices by moving a joystick in front of a computer monitor to obtain a fruit juice reward. It is not unusual for NHPs to remain focused and busy on these tasks for several hours on a given day. During these periods, the animals sit in a custom designed 'primate chair' (a Perspex box) in a natural position.
When performing brain recordings, we restrain the animal's head movements by connecting an implanted head holder to the primate chair. For experiments where this is required we slowly and carefully train each animal. This can take several months.
Once trained the NHPs are visibly relaxed and will fully engage in their tasks. Indeed, the animal's comfort, cooperation and engagement are essential if we are to obtain useful scientific results. An animal that is not comfortable, or ill, will not perform their task properly; thus, it is in our interest as researchers to make sure our animals are comfortable. If a researcher detects lack of comfort in an animal sitting in the primate chair in the experimental room, we stop and give it a break. We then search for and eliminate the reason for their discomfort. If the situation cannot be remedied during that day's session, we stop the procedure for the day, give the animal any remaining liquid, and return the animal to its holding cage. We do not test the animal in the experimental room before the reason for the discomfort has been eliminated.
During our experiments we control each animal's access to food and fluid. However, when we test the behaviour or record from and/or stimulate the brains of our animals, we ensure that each animal always receives its daily fluid and food requirements, and we make sure particularly attractive foods, such as fruits are provided. Basic, nutrient, dry food (NHP chow, dried fruits, nuts) is available at all times in the home cage. We carefully monitor each animal's weight so we can adjust the feeding regime if necessary. We provide free food and water access at least one day every week, and we give our animals week(s)-long breaks every few months.
In order for us to be able to record the activity of neurones in the brain we have to undertake several surgical procedures on our NHPs. These surgeries normally take about 5 to 9 hours during which our animals are fully anaesthetised (asleep and unable to feel pain). Just like humans undergoing surgery in a hospital, our surgery is performed under fully sterile conditions, and with subsequent pain relief provided as advised by the veterinarian. Brain recordings require each animal to undergo surgery during which metal implants are attached onto the skull. The first surgery is often a metal restrained post. Months later a small chamber may be implanted onto the skull. In a small number of cases, an additional surgery or two may be required to repair an implant. Following surgery, we often locate brain areas we intend to study deep in the brain by using one or more x-ray sessions during which the animals are under sedation anaesthesia. During these sessions we record the activity of neurones, which enables us to specifically identify the brain centres that respond when we touch different parts of the body (which otherwise would disturb an awake animal). We also sedate our animals every few months in order to thoroughly clean their implants. This means each animal will undergo full general anaesthesia for a surgical procedure up to a maximum of four times in its lifetime, plus several periods of sedation anaesthesia for the shorter procedures such as implant cleaning.
We record the activity of individual nerve cells in fully conscious (awake) animals. This is done by inserting

microelectrodes (extremely fine needles, less than one fifth of a millimetre) through a small skull opening within the implant chamber into a specific brain area. The stability of these recordings requires head restraint. These sessions normally last up to 4 hours, exceptionally up to 5 hours, after which the head restraint is removed and the animal returned to its home cage. The head restraint schedule will continue for up to 30 months, and in rare exceptions 48 months. These recording with head restraint are performed up to 5 days each week for several months, after which the animal is given a one- or two-week break before returning to testing.
Electrode insertion does not cause the animal pain, because the dura, which is the tissue covering the brain, has lost its ability to detect pain, and because there are no pain receptors inside the brain. Each animal is likely to experience occasional mild discomfort when sitting for extended periods and after having experienced fluid restriction in the hours before testing starts. However, the occasional discomfort is mild and not sufficient to stop the animal from making sophisticated decisions when offered different choices in order to obtain a reward. The way the rewards are presented is carefully designed to enable the researcher to detect the lack of comfort during the experiment and in the recorded data (for example the NHP will make poor choices or no choices at all). With more intense discomfort that agitates the animal or affects its voluntary choices, we search for the reason and remedy it immediately, or return the animal to its holding cage as described above.
We also inject very small volumes (microlitres) of solutions containing molecules that will label the nerve cells we are interested in the specific parts of the brain. The use of these labels helps us identify the exact neurones for recordings or stimulation using small optical fibres (less than one fifth of a millimetre). Such injections may be done under sedation, in which case this will add to the total number of sedations an animal receives.
Surgery or inserting electrodes is not without risk. Animals can develop brain infection or oedema (swelling), surgical stitches may open, paralysis or mild muscle weakness on one side of the body may occur, or mild and almost undetectable seizures have been seen in rare cases. We treat these conditions with antibiotics, anticonvulsants, pain medication, and other medication as advised by a veterinary surgeon. If affected animals do not show signs of improvement within 12 hours, they are humanely killed. In addition, if the animal has not recovered within 12 hours in cases of pain or complete limb paralysis, 24 hours in cases of brain oedema, or 48 hours in cases of systemic infection and wound healing, the animal is humanely killed. Any animal that develops more than mild, localised and transient seizures that require medication using antiepileptic drugs for more than 24 hours will be humanely killed. Complete paralysis has never been observed and is unlikely to occur and would result in immediately humanely killing the animal.
In summary, each animal will undergo behavioural training for about 1 year after which it will undergo neuronal recordings for up to 30 months, and in rare exceptions 48 months. At the end of this time, each animal is killed humanely.
A retrospective assessment of these predicted harms will be due by 30 March 2024

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

To understand higher brain function and provide crucial information for improving the treatment of human disorders, we need to investigate biological signals in the intact working brain. Understanding individual cells is the key for understanding higher brain function because individual cells are the basic units of the brain that process information. Only by doing this in NHPs can we understand how individual nerves function in relation to behaviour at a level found in humans.
Computer simulations, tissue studies and human brain imaging do not permit researchers to study the activity of individual brain cells.
Other animals such as insects or rodents do not have the complex human like behaviour, the highly developed brain structures of interest (e.g. frontal cortex and connected structures), do not permit sufficiently precise

distinctions between reward, decision making and movements, and thus do not allow identification of reward and decision brain cells. Despite some progress in investigating the activity of brain cells in humans, systematically controlled experiments still require the use of animals.
A retrospective assessment of replacement will be due by 30 March 2024

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

Our study design is based on the need to produce reproducible, relevant and scientifically robust data. For example, we compare the activity of a neuron after a reward against the activity of the same neuron before the reward. We can also compare a neuron's activity between several rewards.
We also use modern machine learning tools ('decoders', 'classifiers') that predict the animal's choice by looking at the responses of brain cells to previous rewards, which demonstrates that the brain cell is involved in the decision process. We use computer models to translate behavioural theory into efficient experimental design, which limits the number of experimental tests we need to perform. We have developed advanced computer simulations that help us to understand and predict how behaviour and brain activity are related. We use these tools to make our experimental designs and research questions more specific and informative, which limits the necessary amount of experimentation.
Each neuronal study requires data from 60 or more neurones from a single animal, and a minimum of 2 animals to assure reproducibility across individuals. Sometimes 3 animals are necessary to obtain more robust results. On occasion we have found that the behavioural tests also require 3 animals.
To ensure that we make the best use of the data we gather in these experiments, we analyse the data using statistical methods including regressions, general linear models and machine learning tools. We also use these data to help us to plan and carry out the experiments in a more appropriate and efficient way, using the least number of animals and reducing the duration of behavioural training.
Our experiments are based on knowledge of formal, well established theories of how humans and animals learn and make choices. This theory based approach helps us address more effectively the scientific questions being investigated.
Our related human brain imaging studies help us select the brain structures for investigation at the single cell level in the current animal project.
Together, these approaches help us to avoid unnecessary experiments and ensure we focus only on those parts of the brain that are relevant to the behaviour under investigation. **A retrospective assessment of reduction will be due by 30 March 2024**

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We work hard to use methods that are the most refined, and we spend considerable time, effort and manpower

changing and enriching our animal accommodation and our primate chairs. For example, at least once per month, an aquarium with animated artificial fish is placed in each room for the animals to watch. We locate the aquarium in the room where the animals live but outside their reach. We also show videos that we believe our NHPs find interesting, as judged by their behaviour.
Incidents of fighting can occur as part of the normal hierarchical/dominance structure of NHPs. We supervise special interaction time for animals that need to be separated from others, either temporarily or longer term. The supervision sessions last for several hours each day and involve our experienced laboratory technician being present inside the holding room throughout that time. An animal that needs to be separated from others over longer periods will be able to see, hear and communicate with the other animals living in the same room and, if socially compatible, will have direct interactions for grooming and other social activities under supervision.
We provide layers of wood shavings on the floor of the animals' cages in order to mimic aspects of NHPs natural foraging environment. The animals search for their daily diet (foraging), which also alleviates potential boredom.

We change the fruits and other foods frequently, which appears to keep the animals interested and stimulated. We add to this a variety of more interesting combinations of vegetables.
We use variable sized home cages which allows us to house groups of different numbers of animals. Inside the cages we provide hiding boxes, use video systems to monitor the animals' behaviour and interactions; supervised grooming periods serve to provide social encounters in socially unstable animals. We believe that the combination of all of these measures makes the life of our animals more interesting and reduces the potential for aggression and fights.
We improve the surgical techniques and materials we use and now use an even more tissue friendly material for head implants that binds well with the animals' natural tissue. We make a point of learning and evaluating different methods from new laboratory members joining us from other primate laboratories. We have improved our surgery techniques over the years through experience gained from working and talking to orthopaedic and plastic surgeons.
In addition to these specific points, we ensure that our choice of animal species, care and welfare methods, and scientific methods are the most refined by current standards as follows:
We follow the published guidelines of the NC3Rs and, together with our animal carers and technicians, regularly attend workshops and training courses on animal welfare.
We continue to refine our experimental and scientific approach. We interact and collaborate with other neuroscientists, biologists, medical doctors, statisticians, and data modelling experts nationally and internationally, to exchange knowledge and information gained from work on humans, different animal species and computer simulations.
We continue to remain up to date with the increasingly delicate details of psychological learning theories (so-called Rescorla-Wagner Theory of animal conditioning, and efficient 'Temporal-difference Reinforcement Learning' solving the Bellman value function for optimising reward) and economic decision theories (so-called Von Neumann - Morgenstern Expected Utility Theory, Kahneman & Tversky's Prospect Theory, and Revealed Preference Theory). These theories contribute conceptual frameworks for the experiments and provide more stringent approaches for addressing the scientific questions of reward processing and economic decision-making.
We look for answers to our scientific questions using a number of methods. These include advanced behavioural tasks, single-neurone recordings and computer modelling. In addition, we supplement our animal studies with human brain imaging. We do all this to ensure that our animal experiments address questions that can be answered by single-neuron recordings in NHPs; in other words, we make sure that our animal data will be relevant for humans and their pathological conditions. We refine all of these approaches so that our research can be rigorously peer reviewed by research grant awarding bodies and by the editors of the journals in which we publish our scientific data. **A retrospective assessment of refinement will be due by 30 March 2024**
The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

136. Neutrophils in infection and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

infection, neutrophil, macrophage, inflammation, cancer

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project will study the mechanisms that regulate inflammation focusing on neutrophil and macrophage responses and how they are affected by changes in hematopoiesis during infection, chronic inflammatory disease and cancer.

A retrospective assessment of these aims will be due by 30 May 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Neutrophils are crucial for immune defence but are also driving immune pathology when they become deregulated. Severe infections, cancer and atherosclerosis are the major drivers of premature death worldwide. Neutrophils play a critical role in all of these diseases. Understanding how neutrophils protect against infection and more importantly how they are implicated in sepsis, cancer and atherosclerosis and other inflammatory conditions will provide new avenues of treatment for diseases that remain largely untreatable.

What outputs do you think you will see at the end of this project?

This project aims at uncovering novel mechanisms that drive sepsis, cancer and chronic inflammatory disease that could be targeted therapeutically in order to improve the lives of patients suffering from these diseases. In addition, it aims at addressing fundamental questions about the functions of neutrophils in immunity and disease. This work will be published in a number of scientific research publications and collectively present an inter-connected body of work that will advance our understanding of the mechanisms that fine-tune the production and function of neutrophils and regulate their interactions with the other parts of the immune system.

Who or what will benefit from these outputs, and how?

In the short-term (2-5 years): academics, researchers in academic institutions and the pharmaceutical industry.
In the long run (5+ years) inflammatory disease, infection and cancer patients, clinicians and the general public.

How will you look to maximise the outputs of this work?

Presentation in national and international scientific meetings and seminars at other research institutions.
Publication of high impact research papers, reviews and book chapters.
Collaboration with other basic and clinical researchers.
Disseminations via public engagement activities.

Species and numbers of animals expected to be used

- Mice: 25000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We work with mice as they are well-characterized model organisms to study the immune system and model human infection and disease. In addition, countless critical genetic resources have been developed in mice that are invaluable for our work.
Nearly all of our immunological challenges are tested on young adult mice which have robust and mature immune systems.

Typically, what will be done to an animal used in your project?

The protocols in our project employ a number of procedures such as injections or administrations of microbes, immune or tumour cells of small molecule substances, intravenously, orally or intratracheally. Moreover, some mice receive an altered diet, such as a western style high fat diet. In one protocol mice will undergo a minor surgical procedure to inject tumour cells into the pancreas. Depending on the experimental design experiments with microbial challenge last approximately 1 week. some mice may be infected on multiple occasions with small doses of microbes to evaluate the effect of chronic low grade exposure on tissues and immune cells. Some mice may be infected with higher doses that will cause pneumonia or a skin abscess. Other mice may receive microbes systemically that cause septic shock in order to understand the mechanisms that promote the condition as well as the mechanisms that protect against its onset. Tumour challenges last approximately 2 weeks, arthritis models 10 days and atherosclerosis dieting typically 6-12 weeks. Animals may also be induced to develop arthritis that spontaneously resolves after 2 weeks.

What are the expected impacts and/or adverse effects for the animals during your project?

Infections typically cause weight loss and some transient discomfort characterised by reduced activity. Skin infections may cause abscesses that in some cases may ulcerate resulting in the animals having to be sacrificed. In more severe cases such as sepsis models, mice may experience systemic inflammation with some pain, lethargy, drop in body temperature and respiratory distress, which will be endpoints in the experiment. However, monitoring the temperature allows us to cull the animals before they reach these severe symptoms if meaningful results can be obtained in earlier stages of the condition. Tumours do not usually cause visible symptoms but some weight loss may occur. Large metastatic tumours may cause more significant weight loss and respiratory problems which will be endpoints in our experiments The atherosclerosis model does not cause any visible harm aside from weight gain and vascular plaques that are asymptomatic. The arthritis model will cause some limb swelling and may impact on mouse mobility to some degree.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of our protocols are of mild or moderate severity. Only one protocol employing a model of microbial sepsis is classified as a severe protocol as mice are expected to develop symptoms associated with systemic hyperinflammation. However, the mice will be closely monitored and will be sacrificed as soon as these symptoms begin to appear.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 30 May 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our projects investigate the functional and mechanistic basis for inflammatory diseases. While certain aspects can be addressed using in vitro cultured experiments, to establish the relevance in vivo and to dissect the events that drive these diseases functionally in in vivo requires the use of animal models of disease, particularly since none of the diseases we are studying can be fully recapitulated and modelled in vitro using organoids. Sepsis is a complex disease implicating several different organs and cell types. Similarly, atherosclerosis and the interactions of immune cells with tumours must also be examined in their native in vivo environment.

Which non-animal alternatives did you consider for use in this project?

We are employing human primary neutrophils and other myeloid cells to conduct many of our mechanistic experiments before proceeding to in vivo validation. We are also conducting descriptive studies with human clinical samples of sepsis and atherosclerosis. This approach reduces the number of mice we use in our projects. However, for certain projects as in sepsis we have relied on mouse experiments guiding subsequent mechanistic in vitro studies. The only real alternative to in vivo studies are organoids but they are not applicable to the diseases we are studying.

Why were they not suitable?

All of the aforementioned approaches are complementary to in vivo experiments, but unfortunately cannot replace animal work given that the questions that we are investigating have to be examined in the native disease context.

A retrospective assessment of replacement will be due by 30 May 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Pilot experiments and biological replicates
When setting up a new experiment, rather than using large groups of animals and having to repeat these large-scale experiments to refine the experimental parameters, we start with very small groups (23 animals /group) and test several parameters we predict to be important for optimization.
Subsequently, we follow up the selected conditions that provide good experimental data with additional experiments consisting of small groups that we add to the original study until we reach statistical significance. This phased sequential experimental design reduces the overall number of experimental animals used in our

studies since it limits the number of animals that participate in studies under suboptimal conditions.

In addition, we keep experimental groups small and divide mice over a larger number of independent experiments (biological replicates) in order to obtain better statistical significance from a smaller total number of experimental mice.

Power analysis
Prior to designing experiments we use published data and our own past experience to set the appropriate sample sizes. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 20%.
Imaging
We are also implementing imaging techniques that in many cases are non-invasive and allow us to monitor the progression of infection, inflammation and tumour growth, without sacrificing animals. In this manner we can constantly monitor experimental animals and select optimal timepoints to terminate experiments rather than using multiple animal cohorts sacrificed at different timepoints in order to capture the best timepoint for measuring parameters. This approach minimizes the premature termination of experiments which would require unnecessary repetition and prevents unnecessary suffering by not allowing experiments to proceed beyond the optimum time point.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

As specified above, we conduct small scale pilot experiments to guide power calculations prior to expanding our studies. In many cases long term experiments are performed with in smaller groups and in a phased manner, allowing us to increase the total sample size progressively until the required statistical power is achieved. The early results of the magnitude of changes between experimental groups are filtered through online power calculation tools to estimate accurately the sample sizes required for publication. Through small sample sizes and pilot experiments we can also refine variables like microbial and treatment doses for subsequent experiments

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

1. Breeding: We monitor the breeding of our mice closely to ensure that only breed the number of animals we need for experiments without breeding excess animals that will not be used in any experimental protocols.

2. Multiple uses of tissues, future proofing: From each experiment we collect the maximum number of tissues, even though we may not need them all for the purposes of the present experiment. Given that we employ standard experiments in many of our projects, we keep frozen tissues and samples from all these mice and organise them in a database. This allows us to easily access the tissues in the future without needing to replicate the experiment with new mice. This practice has reduced the number of experiments we have been conducting in the past.

3. Small pilot studies always inform our group sizes, as well as avoid unnecessary large scale experiments that do not show promising results in the small-scale pilot studies. If pilot studies fail to demonstrate signs that experiments may yield interesting data, then no follow up large scale experiments will be conducted and projects will take other more promising directions.

A retrospective assessment of reduction will be due by 30 May 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will employ several murine models of pulmonary and systemic infection using fungal and bacterial pathogens. We will also employ two models of sterile chronic inflammatory disease: murine atherosclerosis using the administration of high-fat diet and a transient model of rheumatoid arthritis using injection of auto-reactive antibodies against collagen. Finally, we will employ several models of primary and metastatic tumours.

Why can’t you use animals that are less sentient?

These murine models are optimised to cause the least amount of suffering. They last anywhere from 24 hrs to several weeks and therefore the animals cannot be anaesthetised for this period of time. However, they are temporarily anaesthetised when undergoing certain procedures. Murine models of disease are very similar to human disease and recapitulate many of the attributes and mechanisms of pathology observed in human patients. They also have a very similar immune system with that of humans that is extensively characterised. In addition, over several decade a multitude of genetic tools and reagents that are specific for mice have been developed that are essential for our research.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Over the years we have refined several of our protocols, particularly by fine-tunning the rate of monitoring and infection doses in our severe sepsis protocol which allow us to predict relatively accurately when WT and GA mice will develop sepsis. We have also refined the breeding pairs needed for maximal use of mice in experimental protocols. These are constantly adjusted according to experimental needs. The time-courses for atherosclerosis experiments have also been well characterised from our prior work, allowing us to estimate with accuracy the length of time allowed to obtain the degree of plaque formation needed for specific experiments. We have also refine our anaesthetisation protocols which reduced the recovery time for mice and reduced the appearance of unexpected symptoms in response to infection.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We are aware of NC3Rs. We also discuss with colleagues in other research groups new improvements that lead to refinement. In addition we follow the literature and improvements in commercial reagents in a constant search for more efficient and better refined alternatives. This has led us to improve our arthritis model which is now 100% penetrant, requires fewer injections and much less time and is much more predictable and consistent than

the traditional immunisation protocol.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our institute regularly distributes newsletters and holds seminars to inform us on 3Rs and ways of improving our methods reducing the number of mice we use and refining our techniques both en mass and at a personal level. We also regularly seek advice from our veterinarian on how to improve our procedures.
A retrospective assessment of refinement will be due by 30 May 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

137. New biomaterials for healing of non-union bone defects

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Bone regeneration, Non-union fractures, Biomaterials, Growth factors, Stem cells

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this research project is the development and testing of advanced biomaterials, seeded with or without stem cells, to enhance the effects of growth factors (GF) during tissue healing. The use of microenvironments that direct the effect of the GFs in tissue healing, or carriers that efficiently control the local delivery of these biological molecules in the site of injury, will generate a platform to enable tissue repair, demonstrated on non-union bone defects in mice.
In particular, we want to assess the translational potential of our biomaterial systems in bone healing, as a novel, robust and safe system to promote bone regeneration in non-union bone defects.
This project will be developed under the principles of the 3Rs described in this licence and all animals used under this licence will be reported.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Non-union fractures, arisen from trauma or disease, are growing in occurrence due to an ageing population and are a major burden for the National Health Service (NHS). In the UK there are approximately 850,000 new fractures each year. A rate of 5–10% are non-union fractures and the treatment costs to the NHS, associated to this kind of fractures, have been reported to range between £7000 and £79000 per person. The development of a platform that enable tissue repair, in an efficient and safe way, will significantly improve societal health by increasing regenerative potential while reducing the life-threatening issues and high cost associated with the use of current high growth factor doses.
This research work will provide a pathway for the translation of this platform into a variety of clinical applications in tissue repair and regeneration, and it is important not only from the point of view of human medicine but also considering animal health, as experts from veterinary schools and practices report many traumatic injuries including non-healing bone defects.

What outputs do you think you will see at the end of this project?

We expect our proposal to overcome key barriers to translation of bone regeneration technologies into a clinical application. We will obtain essential information about the efficacy of our biomaterial systems to promote bone regeneration in animal-based models and treat non-union fractures, which will be critical in the clinical translation and commercialisation of our technologies for bone repair.
We expect to publish the results arisen from this project in high impact journals, which will lead to establish new collaborations and secure new research grants.

Who or what will benefit from these outputs, and how?

The proposed research will generate a unique platform to promote the regeneration of non-union bone defects, using biomaterials that maximise the efficacy of GFs in tissue healing. These studies will provide a pathway for the future translation of the developed technologies into the clinical practice.
This research is important from the point of view of human medicine but also considering animal health, as experts from veterinary schools and practices report many traumatic injuries including non-healing bone fractures.
We envisage results of this project to support technologies that can be translated to veterinary and human clinical practice to promote bone regeneration in complex fractures (e.g. fractures with infections). We believe

that the treatment of these fractures is a stepping stone towards more demanding and prevalent conditions such as osteoporosis and bone tissue tumours.

How will you look to maximise the outputs of this work?

The availability of this animal model will allow us to establish new collaborations with experts in the field of bone regeneration.
The results arisen from this work will be presented in international conferences (e.g. annual meeting of TERMIS) and submitted for publication in high impact journals. Outcomes of our work within our previous licence with this model already gathered the attention of clinicians, which led us to successfully treat our first veterinary patient using our technology. This work gathered the attention both in press and TV and, up to date, has allowed us to treat more than 10 clinical cases of dogs and cats.

Species and numbers of animals expected to be used

- Mice: 300 NSG mice, 200 C57BL/6

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice have many similarities to humans in terms of anatomy, physiology and genetics. Adult mice reproduce quickly, easy to handle, and of appropriate size.
We expect to use C57BL/6 mice, a very common line of mouse used in lab research, in up to 8 weeks long experiments. In those cases where human mesenchymal stem cells (hMSCs) are implanted we will use immunocompromised mice (e.g. NSG strain, commercially available) in order to avoid rejection issues and ensure the survival of hMSCs after implantation.
Since this project will evaluate the potential of different material systems to promote bone regeneration, adult mice will be used in order to prevent the interference of the spontaneous bone growth of animals in a more immature life stages with the outcomes about the efficacy and efficiency of our technology.

Typically, what will be done to an animal used in your project?

All surgical procedures will be performed under aseptic conditions by trained personnel.

a) Mice will be anaesthetised and analgesia will be injected.

b) The radial bone of the mice will be exposed and a bone segmental defect will be created by using a double-blade bone cutter.

c) A polyimide implant tube, loaded with the material to be tested, will be implanted in the area of the defect, fitted to both bone ends. Then, the wound will be sutured.

d) Following implantation, mice will be monitored continuously until fully recovered from anaesthesia and ambulatory. Analgesia will be provided through the food up to few days after surgery.

In those cases where mesenchymal stem cells (MSCs) are implanted, and in vivo cell tracking will be performed, a dose of luciferin will be injected into the mice and animals will be imaged before allowing the animal to recover from anaesthesia (t=0).

e) At different time points, up to 8 weeks, mice will be anaesthetised, and a dose of luciferin will be injected into the mice. 20-60 min after injection, animals will be imaged. Mice will be monitored continuously until fully recovered from anaesthesia and ambulatory.

f) At the endpoint of the experiment, 8 weeks after implantation, animals will be humanely killed by Schedule 1 protocols.

What are the expected impacts and/or adverse effects for the animals during your project?

- Some animals (less than 15%) can show light weight loss, some swelling in the arm, limping and/or attention to surgical area (lameness) - Up to few (2-3) days after surgery.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All surgical procedures will be carried out using aseptic techniques. All animals are expected to make a rapid and unremarkable recovery from the anaesthetic.
The level of severity for each procedure is as follows:
- Surgery to create a critical-size bone defect - Moderate severity in all the animals (100%).

- Luciferin injection and animal-based imaging - Mild severity in all the animals (100%).

The experiments will be done following the 3Rs and any unexpected adverse effect or severity will be reported.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The research proposed in this project is in the field of tissue engineering; it is related to bone repair strategies and, consequently, can be considered to be medical research. It involves chemical, biological, biophysical and biomechanical factors that are not possible to reproduce using lab-based experiments. Therefore, animal experiments are a crucial element of the project, to validate the efficacy of the platform and as an essential pre-clinical validation of the new technologies.

Which non-animal alternatives did you consider for use in this project?

We use lab-based platforms to test the biological response to the new biomaterials. We maximise the use of lab-based experiments in order to reduce the number of animals to be used. In this sense, the lab-based platforms will be used to optimize the composition of the material systems, so that only a selected number of material compositions will be evaluated during the lab-based experiments.

Why were they not suitable?

The responses of cells and proteins properties to the implanted materials used are not possible to fully reproduce in the lab, and often different in an animal or in a cell culture. Therefore, animal experiments are a critical step on this project, to validate the efficacy of the platform in an environment similar to a human body, and are an essential validation in these technologies before a clinical application.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the number of animals based on our previous animal-based studies and our current ongoing projects involving the use of animal experimentation. We have also engaged with statisticians and we will seek their advice to optimally design our experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We expect to perform these experiments for a minimum number of material compositions of the supporting scaffolds, depending on the final outcome from the lab-based cellular experiments.
To keep the number of animals necessary for these experiments as low as possible, minimum number of animals per each group are calculated by using a power calculator (e.g. PS Power and sample size), considering an adequate estimate of standard deviation as well as a statistically significant number of replicates from previous studies using this model, in order to achieve statistical relevance and meaningful results. We will seek advice from mathematicians and statisticians to optimally design each experiment.

What measures, apart from good experimental design, will you use to optimise the number of animals you

plan to use in your project?

Before starting the animal experiments, we have planned extensive research on cell cultures to select the most promising systems, e.g. those inducing higher cell viability, adhesion and/or osteogenic differentiation. In this sense, the lab-based platforms will be used to optimise the composition of the material systems, so that only a selected number of material compositions will be evaluated during the animal-based experiments.
Since variability between the outcomes from lab-based to animal-based studies can be found, we will include animal-based pilot studies with reduced number of experimental groups that will allow us to select only the most promising systems to be fully evaluated.
Non-invasive animal-based imaging techniques (e.g. IVIS Spectrum In Vivo Imaging System) will be used to track the viability of the implanted cells during the healing process, without severely hurting the animals and making it possible to get periodical information using the same imaging test in the same animal over time. This will drastically reduce the number of animals to be used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

A segmental radial bone defect model in mice will be used in this project. We consider the model of critical-sized, non-healing segmental bone defect in the murine radius as a clinically-relevant and yet least invasive model to evaluate the potential of implanted biomaterials to induce bone regeneration. We strongly believe that non-healing models are necessary as they are rigorous test-beds for bone repair strategies, and this model has been successfully used in our group.
This bone repair model has significant and particular advantages for this research:

- 2.5 mm defect does not spontaneously heal during the course of the experiments providing a rigorous critical-sized model to evaluate bone repair strategies.
- The smaller animal model allows for easy use of animal-based imaging approaches (e.g., IVIS).
- The ulna provides sufficient stabilisation of the defect and no fixation plates/hardware is required, thereby considerably simplifying the surgical procedure, reducing the risk of infection, and at the same time reducing animal pain. Therefore, this model allows for efficient screening of several experimental conditions, a major advantage over the rat calvaria and segmental femur defect models that were previously used, and also considerably reduces pain during recovery after surgery.

Why can’t you use animals that are less sentient?

Mice are used due to their anatomical, physiological, and genetic similarity to humans. They have a short life cycle and reproduce very quickly.
Regarding the life stage; since this project will evaluate the potential of different material systems to promote bone regeneration, adult mice will be used in order to prevent the interference of the spontaneous bone growth of animals in a more immature life stages with the outcomes about the efficacy of the treatment.

To be able to evaluate bone regeneration for a defined period of time after implantation, all surgical procedures will be performed under general anaesthesia with recovery.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Following the Home Office advice, the aseptic conditions during the surgeries have improved significantly in order to guarantee the animal welfare. A different set of sterile materials (including sutures, drapes, surgeon gloves, etc) and surgical tools is used for each animal. New extra sets of surgical tools have been purchased for this purpose, including the custom-made bone cutters. The use of glass bead sterilisers to sterilise instruments between animals has been replaced by the use of the autoclave.
At present, the animal facility provides us with a technical assistant who has already experience assisting our surgeries, which helps to reduce the duration of the surgeries.
Where mutant immunocompromised mice (e.g., NSG) are used, they will be maintained in a suitable barrier environment in order to prevent any unwanted infections.
Analgesia will be provided by intra-operative administration and post-operative delivery in the water.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All the animal experimentation will be performed by following the Guidance on the Operation of the Animals (Scientific Procedures)
Act 1986.
I undertake responsibility for the overall implementation of the programme of work and for ensuring that the programme is carried out in compliance with the conditions of the license (including the principles of the 3Rs).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our group and collaborators have been extensively working in the field of bone regeneration for many years and have a wide expertise and up-to-date knowledge of the current animal models used for the purpose of this project.
We are also in continuous contact with the staff from the biological services (NTCO and NVS) and we have implemented advances effectively following the Home Office advice.

Home Office

NON-TECHNICAL SUMMARY

138. New cancer drugs targeting the DNA damage response

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cancer, DNA damage response, Bone metastasis, Cancer treatments, Multiple myeloma

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To validate novel compounds targeting the DNA damage response for the treatment of cancer and effective therapeutic combinations.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cancer is responsible for over one quarter of deaths in the UK. Many chemotherapies currently in the clinic are good at killing cancer cells, but they also kill normal healthy cells.
Cancer cells duplicate themselves much faster than heathy cells. As they do this, they make many mistakes in their DNA (the genetic material that encodes life). By stopping cancer cells from being able to repair their DNA, they will die. Many existing chemotherapeutics work by blocking this pathway, including 5-fluorouracil and methotrexate. Unfortunately, this process is important for all cells, so these treatments also kill healthy cells.

We have identified some parts of this process that are unique to cancer cells and have developed new compounds to block them. This means that unlike the drugs that are already in the clinic, our new compounds selectively kill cancer cells, without killing normal heathy cells. We have shown this in cell culture and in a mouse model of leukaemia. We have also identified a number of cancers that are sensitive to these compounds in cell culture, including leukaemia, myeloma, colorectal cancer, lung cancer and breast cancer, but these new compounds are likely to be effective in many other cancers.

What outputs do you think you will see at the end of this project?

The major outcome of this work will be identifying a clinical strategy for translation of novel cancer therapeutics into patients and will directly influence phase I clinical trials as a mono or combination therapy. This work will be published in frontline journals and will provide valuable insights and new information about these targets and their role in cancer. Once we have published our findings and protected our new drugs (e.g. through patents) the drugs developed in this work programme will be shared with other researchers who think they may be useful for the treatment of other diseases.

Who or what will benefit from these outputs, and how?

In the short-term, this work will benefit the scientific community by providing valuable knowledge on the DNA damage response in cancer and the roles of individual proteins. It is likely that the targets investigated here will also be implicated in other diseases, and the novel compounds will be made available to other researchers for further exploration.
In the mid-long-term, the ultimate benefit of this work will be to cancer patients. During this project we identify biomarkers to predict sensitivity and resistance to the compounds, for identification of patients who are likely to respond to the treatment.

How will you look to maximise the outputs of this work?

We will collaborate with industry and academic partners for the safety and toxicity testing of these compounds, who are better equipped to perform these experiments. This will streamline the process of taking new compounds through pre-clinical testing, allowing us to more quickly move forward promising compounds into clinical trials. We have close ties to the NHS through which we will be able to coordinate clinical trials. We will disseminate our findings at conferences and in frontline journals and where possible include negative data in

these.

Species and numbers of animals expected to be used

- Mice: 2900

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use mouse models of cancer as these are well established, reproducible and reliably mimic much of the disease in patients. Many studies will use human tumour cells, to most reliably replicate the human disease. However, implantation of human tumour cells must be done in mice with a deficient immune system. For some studies it will be important to have a fully functioning immune system, for example for drugs targeting the immune system, in this case mouse tumours will be used.
For each experiment, the model used will be guided by studies in cells and patient screening. In each circumstance, the advantages and disadvantages of each mouse model will be weighed and the model that most appropriately addresses the experimental aims, while adhering to the 3Rs, will be used. Where possible we will use tumour cells genetically altered to allow specialised imaging of tumours that allows for tracking of tumour growth over time, to reduce the number of mice needed in each study. We will use mice of an appropriate age for each model that allows for effective engraftment and tumour growth, this is typically a young adult mouse.
We will perform as many experiments as possible in cell and/or organ cultures and screen patient samples (where possible) before we do any experiments in animals. Many cancer therapies are highly effective at killing cancer cells when treated in cell culture, but not as effective when treating a whole animal. This is because of the complex environments and signalling networks within animals that may prevent the therapy from working, as yet this cannot be modelled in a dish or by a computer. For example, sometimes drugs do not get through the body to the cancer very effectively, or the body might remove the drug before it can do its job.
We will use cell/organoid culture and screening of patient samples to identify sensitive cancer types and effective drug combinations. We will combine this with computer modelling to understand as much as possible about how the drugs work before treating animals, e.g. how drug resistance may develop. Studies in mice will only be performed after this to show that the treatments are effective in a whole animal model, and also to identify which new or approved drugs should be used in combination.
Studies will be designed in line with the NCRI Guidelines for the welfare and use of animals in cancer research (Workman *et al*. 2010). Throughout the study we will re-evaluate the models used in line with the 3Rs (replace, reduce, refine), to ensure we are using the best and most ethical animal models and where possible use non-whole animal models.

Typically, what will be done to an animal used in your project?

Some animals will be injected or implanted with cancer cells. Where possible, this will be done in the way that most accurately mimics with disease in patients (e.g. injected into the blood for blood cancers, or into mammary tissue for breast cancer), sometimes this will require a surgery under general anaesthesia. In some cases, tumour cells will be injected under the skin as this provides easy access and monitoring of the tumour, and avoids complex surgical procedures.
Some animals may be fed a modified diet for a part or throughout the study as this can improve how effective some therapies are in reducing tumour. Some animals will be fed or injected with compounds that we are

developing to treat cancer, or a placebo, this is usually once a day or once a week. Animals may also be treated with other cancer therapies including radiotherapy. Animals will be weighed at appropriate intervals to monitor weight loss, as this is a sign that they are unwell.
To monitor tumour, animals will be put under general anaesthesia for specialised imaging, this is usually once a week. We will also take a small amount of blood from a vein in the tail once a week to measure markers of response which can be detected in blood. For some tumours, other specialised techniques may be used to monitor the disease. For example, for cancers which cause bone disease we will use micro-computed tomography to scan an affected bone under general anaesthesia.
The duration of each study will depend on the model used, studies using more aggressive tumours may only last 3 weeks, whereas less aggressive tumours can develop over several months. Experiments to study the effect of compounds on normal biology without tumours present, will last for a defined treatment period, usually this will not be more than 2 months. The studies will end when the treatment period is complete, when tumours reach a certain size or when we detect relapse (tumour regrows despite treatment) or metastasis (spread to other organs).
Animals will be humanely killed at the end of the study or when they show signs of becoming significantly unwell, whichever is first.

What are the expected impacts and/or adverse effects for the animals during your project?

Occasionally some mice will experience adverse effects, but provisions will be made to minimise these. For example, the cancer or treatment may make some animals feel sick and they may stop eating or drinking resulting in weight loss, their activity may change or they may look scruffy. We will weigh and check the mice regularly to carefully monitor for signs that they may be unwell. We will also measure the tumour regularly, and not allow the tumours to grow beyond reasonable limits. If the mice lose too much weight and don't recover within 24 hours, seem unwell or if their tumour gets too large, they will be humanely killed.

When blood samples are taken, if too much blood is taken the mice can become anaemic. To make sure we do not take too much blood mice will be weighed and only an appropriate amount of blood will be taken from each mouse on each occasion (https://www.nc3rs.org.uk/mouse-decision-tree-bloodsampling).
The drugs and compounds we will treat the mice with are not expected to have any adverse effects at the doses we will use. The injection or feeding of these drugs will not cause any more than a short discomfort at the time of administration.
General anaesthesia for surgery, imaging or tumour implantation is not expected to have any adverse effects with appropriate monitoring and pain relief. Mice will be closely monitored during anaesthesia and if breathing becomes irregular then mice will be humanely killed.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Animals will experience a maximum of a moderate severity level.

It is expected that 98% of animals will experience a moderate severity, some animals in which tumours fail to grow will only experience a mild severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our goal is to develop new treatments for cancer. To do this, we will perform experiments using cells, patient samples and computer modelling as well as mouse models of cancer. While non-animal models of cancer are very helpful in understanding aspects of biology and how drugs work, unfortunately they do not model the entire body. Many cancer therapies are highly effective at killing cancer cells when treated in cell culture, but do not work when treating patients. This is because of the complex environments and signalling networks within humans and animals that may prevent the therapy from working. As yet this cannot be modelled entirely in a dish or by a computer. For example, sometimes drugs do not get through the body to the cancer very effectively, or the body might remove the drug before it can do its job.
We will perform as many experiments as possible in cells and test patient samples before we do experiments in mice. As much as possible we will replace animal models with non-animal models and we will continue to investigate non-animal alternatives throughout the duration of this licence.

Which non-animal alternatives did you consider for use in this project?

Animal experiments are one key part of this work programme required to achieve our aims, which we will also combine with non-animal models.
We will use 2D and 3D cell culture to study the biology of new targets and to test novel compounds. This will include identifying if the drugs kill cancer cells and how they do this. By screening in cells from a wide range of cancers we will be able to identify sensitive cancer types and 'biomarkers' that can be used to predict which patients will respond well or poorly to the drugs. We will also test our new drugs on tumour samples from patients. We are also investigating the chick embryo assay to see whether they can replace some of our animal models (https://www.nc3rs.org.uk/development-chick-embryoreplacement-rodent-models-tumour-metastasis). This may be useful for some tumour models using human cancer cells. However, limitations to these models mean they cannot fully replace the mouse models here. After performing these studies, we will use mouse models of cancer before doing clinical trials.
Throughout the study we will continue to investigate non-animal alternatives through resources such as the NC3Rs and the Norecopa 3R Guide. Alternatives will be implemented when relevant.

Why were they not suitable?

The non-animal experiments are all suitable models for early investigation of new drugs for cancer treatment, and we will use these to complement studies in mice. However, experiments in a dish or computer are unfortunately not capable of reproducing the effects in a whole organism.
Many cancer therapies are highly effective at killing cancer cells when treated in cell culture, but either do not work or are less effective when treating a whole animal. A whole animal has many natural barriers that can prevent a drug from working the way it would on an individual cell. After entering the body the drug may never reach the tumour or the drug may be broken down and removed too quickly to have an effect. On top of this, tumour growth and spread (metastasis) can also be controlled by other cell types in the body, like immune and bone cells, and these cells can also influence drug responses. Finally, there can also be systemic effects, like hormone levels, that may change the response to the drug. There are no non-animal models that exist that take into account all these factors. However, we will continue to investigate alternatives.
The chick embryo assay can be used to study cancer, but there are limitations to this assay that mean it is not the ideal model for many of the drugs we are testing. Firstly, it cannot be used to study cancer in bone, which form a big part of this work. This model may be useful for some models looking at primary tumour growth.

However, the immune system is important in cancer, and this model lacks an immune system, meaning mouse models offer the models which most closely represent human cancer patients.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals we will used is based on previous quantitative published data and experience.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have planned to use a number of non-animals models (e.g. cell models and screening of patient samples) before any experiments in animals to reduce the number of animals required.
Experiments were planned using using specialised software and best practice guidelines. This includes the NC3Rs Experimental Design Assistant and the Norecopa PREPARE guidelines and the National Cancer Research Institute's *Guidelines for the welfare and use of animals in cancer research* (Workman *et al.* 2010 *Brit J Cancer*).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

If we do not have previous data to estimate the number of mice we should use for an experiment, we will perform a pilot study to estimate the minimum number of mice required. For complex experiments, we will seek advice from specialist statisticians to help in experimental design.
We will use mouse models of cancer as these reliably and reproducibly mimic the growth and spread of cancer in humans. We will use modified cancer cells so we can use specialised techniques, like non-invasive tumour imaging. We will also use other specialised monitoring techniques for specific cancers, e.g. micro computed tomography for cancers that cause bone disease. This will reduce the number of mice required for experiments, by performing measurements over time in the same animal rather than requiring multiple groups to investigate different timepoints. We will also use inbred mice because this minimises variation between mice, thereby reducing the number of animals required.
Where possible we will store tissue that may be of interest to investigate in the future, even if analysis is not planned in the original design. This will minimise the risk of having to repeat a study to obtain more tissue/data. We will also make this tissue available to other researchers upon reasonable request.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use two types of mouse models of cancer to study the effects of new potential cancer therapies.
The first uses human tumour cells or tissue implanted into mice that lack part of their immune system. This is required so that the body does not reject the human tumour cells. The second model, uses mouse tumours implanted into mice with a fully functioning immune system. This is also important as the immune system can influence tumour growth and response to therapy. These models have been extensively used by us and others, so there is a wealth on information available on these models and they are considered to be reliable and reproducible. These models have be optimised to minimise pain, suffering, distress and harm to the animals.
I have over 10 years experience working with mouse models of cancer, and during this time have developed refined mouse models of myeloma. These models are more representative of patients, and use specialised imaging to monitor tumour rather than relying on symptoms of mice being unwell or in pain. Our colleagues have similarly developed refined models of breast cancer, which we will use in our studies. We have extensive experience working with these mouse models, with world-leading techniques for monitoring tumour and bone disease plus analysis of tissues to understand how new drugs work.
We will also use general practices that minimise stress, harm and pain to animals. This includes nonaversive methods for handling mice (e.g. not picking up by the tail), the use of single-use needles to avoid pain from dulled needles and NC3R guidelines on blood sampling.

Why can't you use animals that are less sentient?

To study effect of treatments on tumour growth, metastasis and relapse requires a whole organism model. Mouse models have been extensively used by us and others, so there is a wealth of information available on these models and they are considered to be reliable and reproducible. Mice are also very similar to humans genetically, and many processes in relation to development and growth of cancer are conserved between these species. Mice that lack part of the immune system can also grow human tumours, allowing us to study the effect of new treatments on human tumours.
Some less-sentient species, such as zebrafish, can be used to study specific tumorigenic processes. However, these models cannot be used for studying the effects on intervention on processes like metastasis. They also do not provide the ability to model treatment strategies that are used for patients. For our studies, in order to determine whether our new treatments are effective at treating growth of primary tumours and/or metastasis, mice are the most reliable model and we cannot currently perform these experiments in a less-sentient species. We find mouse models of cancer are most reliable and representative in adult mice and they cannot be used at a life stage where mice are less-sentient.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Tumours will be regularly monitored through non-invasive methods. Mice will also be weighed to check for signs of weight loss that may occur in response to the tumour or treatments. Mice will be regularly checked for signs of being unwell, for example changes to the coat condition, behaviour and movement. We will also continually attempt to identify earlier endpoints for studies, to minimise harm to animals.
Invasive surgeries will be performed under general anaesthesia. Mice will be given pain-relief to manage pain and kept warm while they recover. Mice will be monitored following surgery and any procedures that may have immediate adverse effects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All surgeries will be performed under sterile conditions following best practice guidelines, e.g. LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. Procedures for establishment and monitoring of tumour growth, metastasis and animal welfare will follow best practice guidelines (e.g. NCRI Guidelines for the welfare and use of animals in cancer research, Workman et al. 2010 Br. J. Cancer). We will also consult the NC3Rs website and Norecopa for information on the 3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly consult the NC3Rs and Norecopa websites, the literature and colleagues/collaborators for information on the 3Rs. Any relevant advancements that can be implemented will be used.

Home Office

NON-TECHNICAL SUMMARY

139. Novel molecular targets for the development of new analgesics

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this work is to benefit medical and veterinary pain patients by providing routes to the development of new analgesics that will reduce their pain and improve their quality of life. Pain following nerve damage in patients is especially difficult to treat and represents a real unmet need. We are trying to understand the basic mechanisms involved in this to help to produce new safe and effective analgesic treatments where none currently exist.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Pain relief remains inadequate for many pain states (with limited efficacy and often severe or dangerous side-effects), especially when the pain state involves damage to nerves (neuropathic pain), where specialised molecular changes are triggered. To gain a full understanding of what might be an appropriate intervention, it is important to elucidate how these changes differ from those in inflammatory pain. Effective treatment of chronic pain (especially neuropathic pain) represents a major unmet need with all currently available analgesics showing very limited efficacy (a low percentage of patients experiencing significant pain relief) and unacceptable or dangerous side-effects, that limit how even they can be used. A new, more effective strategy is urgently needed.

What outputs do you think you will see at the end of this project?

We will have new insights into the neurobiology of pain and analgesia, identifying novel molecular targets for innovative treatment strategies. We have a strong track record of identifying new potential analgesic targets over several decades and have recently identified several highly promising and entirely novel new targets, which will be the focus of the current work. Results will be presented at national and international meetings, published in high impact, peer-reviewed journals and made freely available on the internet by Open-Access arrangements.

Who or what will benefit from these outputs, and how?

Academic and pharmaceutical industry researchers will be the short term beneficiaries. In the medium term, the work can potentially lead to the development of novel, more effective, analgesic drugs to deliver improved pain relief and quality of life to clinical and veterinary pain patients. Long term benefits should then include reduced pressure on healthcare systems and a reduction in the economic burden of treatment costs and lost working hours.

How will you look to maximise the outputs of this work?

We will seek maximal distribution of our findings by widespread presentations at scientific meetings and publication in leading Journals, at which we will ensure free Open Access. Both academic and industrial collaborations will be encouraged, to facilitate fundamental understanding and translational development of our findings to benefit patients. We are eager to enter into drug development programmes with our previously established medicinal chemistry and pharmaceutical industry partners (as well as new interested partners), in whatever way will optimise the overall outcome for relieving suffering from chronic pain.

Species and numbers of animals expected to be used

- Mice: 530
- Rats: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rats and mice are the most appropriate for these studies as their nervous systems reflect most of the properties of that in man. Gaining good understanding of fundamental mechanisms of pain processing and its modulation by endogenous mechanisms, that may represent new targets for analgesia, should be readily translatable to application in a clinical context. The vast majority of relevant research literature and understanding relates to work done in these species, so we can readily gain from that knowledge and better predict experimental outcomes. Adult animals are used in the experimental protocols so their nervous systems are fully developed and findings better reflect the situation in adult humans who represent a very large proportion of chronic pain patients.

Typically, what will be done to an animal used in your project?

In the experimental Protocols (1 and 2), animals would normally be anaesthetised for either nerve injury surgery or cutaneous injection of inflammatory agents and then allowed to recover for the relevant period to allow development of the model. For behavioural testing, animals would be habituated to the testing environment, for brief, threshold level paw withdrawal testing and baseline measurements recorded. Animals would then be anaesthetised (generally only briefly) for the administration of selective pharmacological or other agents, designed to interrogate specifically the mechanisms of pain and analgesia. After recovery from anaesthesia, further behavioural testing would be carried out to measure the effect of these agents. Finally, animals would be killed by a humane method, usually then taking tissue for biochemical assessments. Protocol 3 represents solely the breeding and maintenance of externally-derived genetically altered animals.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals in protocols 1 and 2 are expected to recover from nerve injury surgery or inflammatory agent injection without incident. When the models have developed, the animals display a mild degree of hypersensitivity at threshold stimuli levels, in the affected paw and likely some reduced weight bearing on that paw. Grooming and exploratory behaviour are unaffected and occasionally, but not usually, there might be a slight reduction in the rate of weight gain. Behavioural testing is brief and at threshold level, resulting in no discernible changes in the animals' overall behaviour. The specific pharmacological and other agents are expected to have no discernible impact on the overall physiology, experience and behaviour of the animals. No impact/adverse effects are likely to occur in Protocol 3 – standard breeding and maintenance of externally-derived genetically altered animals.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For Protocols 1 and 2 the animals will be moderately affected in a restricted location by the nerve injury or peripheral inflammation. All other interventions represent reliably only a mild severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is not possible to gain valid understanding of the processes of pain hypersensitivity as targets for potential new analgesics without some use of animals such as rodents. This is because processes closely resembling those that occur in man do not occur in simpler organisms with a less highly developed nervous system. Neither can such processes be adequately studied in cultured cells. We use the minimum possible number of animals by carefully planning the experiments and co-ordinating our studies to utilize tissue for corroborative biochemical measurements. For this, we developed new protocols using extremely small samples of tissue, thereby helping to minimise animal use.

Which non-animal alternatives did you consider for use in this project?

Cell lines or computer-based modelling.

Why were they not suitable?

Cell lines are not appropriate as they lack the complex communication between different cell types that would be necessary to gain any meaningful understanding of the complex changes that occur in a mammalian nervous system in response to pain state models and how these changes can be reversed.

Computer-based modelling is not appropriate as far too little is known of the processes and their interactions to even contemplate a meaningful model.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Experiments are carefully designed on the basis of our extensive experience using statistical methods that are widely used to predict the minimum number of animals likely to be needed to achieve clear and significant results. The estimates are based on numbers used in similar, closely related projects over the course of my previous PPL, knowledge of typical effect sizes from the literature, predictive statistical calculations and advice from local statisticians. Further, we ensure that whenever possible all animal tissues are also utilised for sensitive biochemical assays. As a general aim to reduce animal usage we have worked to miniaturise many of our biochemical assays so that only tiny samples of tissue are needed and a range of different questions can be answered using the tissue from a single animal. A small additional number of genetically altered mice are needed as breeding stock.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use online tools and seek advice from local statisticians and NC3Rs Experimental Design Assistant as appropriate.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Beyond best practice at all stages of procedures, we spend a great deal of effort on ensuring that drugs,

antibodies and other reagents are truly specific, so results will be equivocal in interpretation and avoid the need for further experiments to re-validate any findings. Behavioural, pharmacological and biochemical experiments are all carried out by highly experienced investigators who can pre-empt any difficulties and ensure experimental opportunities are used to full advantage.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use rats and mice as these have provided the vast majority of research information in the field and simpler species do not have equivalent pathways that can reflect how pain messages are processed in humans. The literature suggests that rodents provide faithful models of human pain states. We take all possible opportunities to minimise intensity and duration of such models in our work, which generally seeks to validate new improved strategies for relief of pain. All of the tests we use to measure sensory responses are brief threshold-level tests that do not distress the animals. According to 3Rs principles, we have successfully worked to move as many of our assessments as possible to recording responses of tiny tissue samples in biochemical assays.

Why can’t you use animals that are less sentient?

We cannot use less sentient animals as no meaningful results could be obtained that give insights into the aims here, which require investigation of adaptive changes in a complete and fully functional mammalian nervous system in order to understand novel targets for unmet analgesic needs.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We are careful to use local anaesthetic gel (both pre- and post-operatively) to minimise any local discomfort following surgery and we are assiduous about frequent post-operative monitoring of animals to ensure all are well. For behavioural testing, all animals are well habituated to the test scenario and returned as quickly as possible to their home cage. Even minor procedures such as drug injections are only ever carried out following brief anaesthesia in order to minimise stress and discomfort.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We consult the Norecopa PREPARE guidelines as well as the NC3Rs ARRIVE guidelines and their Experimental Design Assistant site to ensure the most refined experimental design.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We regularly look for updates on 3Rs from the NC3Rs site and also consistently attend local advisory update meetings for animal users. New advice is shared with (and implemented by) all in the group.

Home Office

NON-TECHNICAL SUMMARY

140. Novel seeding models to mimic proteinopathies

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Central nervous system, Proteinopathies, seeding human extracts, spreading, cognition - dementia

Animal types	Life stages
Mice	adult, juvenile, neonate, aged
Rats	adult, juvenile, neonate, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The main objective of this project is the development of novel animal models that closely mimic both the underlying pathology and clinical phenotypes associated with neurological and neurodegenerative diseases, which as an underlying pathology have the aggregation of proteins. These models will be utilised to further our understanding of the mechanisms underlying the development and progression of the diseases and testing of potential therapeutic agents.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The present research is vital with the increased life expectancy and a larger proportion of the population aged over 65 years, which in turn has resulted in an increased prevalence of individuals with age related neurological disorders and neurodegenerative diseases including dementia (Alzheimer's, Frontal Temporal Lobar or Lewy body) and Parkinson's disease.

This research will enhance our understanding of brain processes involved in the onset and progression of the diseases which in turn could facilitate the development and testing of new/improved treatments for patients.

What outputs do you think you will see at the end of this project?

- This work will further our understanding of progression of diseases including dementia and Parkinson's disease by studying changes in both neural and behavioural processes.

- The key aspect of this study will be the development of novel more refined animal models of neurological disorders and neurodegenerative diseases including dementia and Parkinson's disease. These models will closely mimic and resemble the pathology, physiology and behavioural symptoms associated with the human diseases whilst presenting with a similar progression that will be more translationally relevant. These models will facilitate the development and testing of new treatments.

- Findings and data from this project will be published in peer-reviewed scientific journals.

Who or what will benefit from these outputs, and how?

The data generated from this research project will guide and inform both our research and could also have benefits for the wider research community. They will have translational value and may be instructive in future clinical studies on how to stratify patient cohorts.

In the long-term it is envisaged that this model will not only facilitate a better understanding of the disease, but it could also be utilised in pre-clinical testing and the potential development and screening of new treatments/compounds by pharmaceutical companies and the NHS and therefore in the longer term it will benefit patients along with their carers/families.

How will you look to maximise the outputs of this work?

The findings and data from this project where possible will be published in peer reviewed journals and will also be shared with the wider research community via presentations at both national and international scientific conferences.

Species and numbers of animals expected to be used

- Mice: 12,200
- Rats: 2,800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

During this project we will try and develop novel animal models that closely mimic human neurological and neurodegenerative diseases including Alzheimer's disease, Parkinson's Disease, Fronto-temporal lobe dementia and Lewy Body dementia.
Mice and rats will be used for the purpose of this project as they have genetic similarities to humans and their genetics and physiology can also be easily manipulated to induce models that resemble the disease profile associated with human conditions. A number of GA models have been developed that are able to model the human diseases and these can be generated in a short-period of time. However, as not all forms of diseases have a genetic cause it is also necessary to induce models that address the 'sporadic' form of the disease onset and this can be done via manipulation of the physiology of the mouse. These seeding procedures may be used in conjunction with GA lines to accelerate their phenotypes and combine familial and sporadic forms of dementia.
Dementia and other degenerative diseases are age-related disorders with symptom onset and development usually evident in elderly patients (over 65 years old), using mouse and rat models allows us to accelerate the disease onset and progression. This can be induced in young adolescent animals with symptom onset and progression evident at approximately 6 - 12 months of age and presymptomatic testing is therefore possible in younger animals. Animals will be kept up to a maximum of 18 months of age.

Typically, what will be done to an animal used in your project?

Novel animal models will be developed using aseptic surgical techniques in order to induce similar pathology in the brain of the animal to that which can be observed in the human disease. This will involve the injection of extracts or substances into the brain regions of interest of the animal.
Appropriate analgesia and care will be given to the animal throughout the surgery and recovery period.
Animals will be given a suitable recovery period following surgery, and time will also be allowed for the pathology to spread within the brain before further assessments of symptoms relating to the disease progression are performed. Assessments may include tests for behavioural symptoms including cognitive impairments, differences in activity, changes in emotional states e.g. anxiety, motor deficits and changes in sensory ability e.g. olfaction. There is a wide range of behavioural tests that could be performed depending on the disease being modelled.
Due to the co-morbidity of neuronal diseases and metabolic disorders in humans e.g. Alzheimer's disease and diabetes, animals may also undergo metabolic assessments to determine if these are also present in the animal model during disease progression.

Following the determination of behavioural and/or metabolic symptoms that correspond with the clinical symptoms associated with the human disease, animals may be given drug treatments to ascertain if these are able to alleviate the symptoms and slow the disease progression.
Behavioural and metabolic (physiological changes) can be assessed independently of one another although due to our knowledge of the disease profiles in human patients the combination of these two approaches may offer a more holistic approach to understanding the disease onset and progression (see Table 1 for protocol summary and complexity).

What are the expected impacts and/or adverse effects for the animals during your project?

- Pain experienced during surgery is transient and will be treated with appropriate analgesic and postoperative care regimes where necessary. Following surgery animals can also display weight loss but again this is typically transient.

- Animals may experience side effects following administration of novel drugs/drug classes but this will be unusual and short-term. Animals will be closely monitored for weight loss.

- No abnormal behaviours are expected as a result of procedures performed under this project, with behavioural changes normally subtle and only observed using specialist and sensitive behavioural equipment.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Severities under this project range from sub-threshold/mild to moderate. The majority of animals will be undergoing surgery and are therefore classified as moderate severity (approximately 80% of rats and mice) due to the surgery itself although they are not expected to suffer further harm following surgery.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are the only option for the analysis of behavioural functions correlated with brain activation. Towards this end, we seek to identify disease relevant biomarkers (relevant for dementia) in the behavioural domain. These will be correlated with physiological recordings (glucose handling etc.), but also with pharmacological profiles of transmitters and enzymes. Inclusion of post-mortem ex vivo analyses provides an enormously powerful tool to determine the mechanisms underlying the disease and to identify novel drug targets and novel treatment options.

Which non-animal alternatives did you consider for use in this project?

There is no suitable replacement or alternative e.g. cell culture, computer model, organelle, nematode, insect

etc. to using the whole animal to study behavioural / physiological changes and related brain function. For this we have opted to work with rodents as their behavioural repertoire is close to human, GA variants are readily available and translation between patient and animal can be achieved.

Why were they not suitable?

The experimental techniques and behavioural assessments which will be utilised in this project can only be performed on the whole animal. Animals are needed in order to understand how changes in brain function can be related to behavioural / cognitive phenotypes observed in neurodegenerative and neurological disorders, and this cannot be achieved using alternatives listed above. Although our laboratory also conducts in vitro / ex vivo based activities using neuronal cell cultures and molecular and biochemical technologies, these preparations alone cannot determine the behavioural objectives of this project.
During this project we will continue to improve and refine our experimental techniques and where possible minimise the number of animals used.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

- The animal numbers used during the course of this project will be the minimal necessary to draw statistically robust conclusions. We typically perform power projection analyses based on our expertise (behavioural, physiological, pharmacological and GA models) whilst taking into account that variables including age (we typically work with 6 weeks to 18 month old subjects) gender, species and background strain can influence sample sizes. Alternative means of power calculation may be derived from the literature.

- Keeping up to date with the latest developments ie monitoring of the literature and attending conferences will ensure that we don't duplicate or repeat procedures with animals where data has already been obtained. An exception to this being when validation of the study via replication of the data is necessary (confirmatory experiments). Our laboratory is currently involved in projects funding reproducibility issues in preclinical studies (both within laboratory and between-laboratories) with an impact on the Reproducibility Network UK and the 3Rs. We draw valuable information from this network in terms of study design and data robustness.

- Given our experience and expertise with the techniques and tests proposed in this licence, we would typically estimate a group size between 12-14 subjects in young subjects with clear genotype/drug-dependent differences of 30%. Otherwise, cohort sizes may need to be bigger and variance will need to be established for novel tests and techniques.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

- This project will employ a within-subject experimental designs where possible allowing repeated testing of an individual animal in a longitudinal design either across different experimental conditions or at different

stages of development/age. Longitudinal designs have been found to reduce animal numbers by at least 50% compared to cross-sectional between-subject study designs.

- Multi-disciplinary/multi-factorial approaches will be employed in order to maximise the information obtained from a minimum number of animals e.g. implementation of a test battery of different behavioural assays.

- At all stages of experimental design, we will follow ARRIVE 2019 guidelines with experimental blinding and randomisation in order to avoid any bias. We are also seeking to implement a novel 'Data Quality System' as pioneered by one of our collaborators.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

- Pilot studies involving smaller numbers of animals will be utilised when exploring novel procedures. The data generated from these initial tests would be subjected to a power analysis in order to inform the appropriate sample sizes for subsequent studies required. However, sample sizes around n=5 are not really instructive for power calculations and can only be a used as a guide.

- Utilisation of advanced and sensitive analysis tools (incl. video analysis software, Matlab routines etc.) can also reduce the number of animals required to make endophenotypes reliable.

- Tissue will be harvested from all of the animals at the end of the study and will be analysed thoroughly (including pathological and metabolic markers) allowing us to attain the maximum amount of data from a given animal.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

- During this project we will develop and use novel animal models that more truthfully mimic the diseases. Advantages of using mice for studying human ageing and disease models include that the genetic and physiological parameters can be easily manipulated and they can be generated in a short period of time.

- We will utilise different GA animal models where necessary to closely mimic the underlying pathology of the diseases and enhance our understanding of both the onset and progression of the different disorders. Most of these animals are normal in their day-to-day home cage behaviour with behavioural phenotypes observed using our sophisticated scientific equipment.

- While behavioural testing (cognition, movement abnormalities, and sensory dysfunction e.g. olfaction) is the main read-out this project, it is difficult to draw conclusions on the underlying mechanisms of disease progression from these tests alone. Therefore we will use additional endpoints, such as pharmacological treatment and metabolic read-outs to determine the mechanisms underlying the disease progression.

- Aseptic surgical techniques will be utilised at all times and will be performed to the HO Minimum Standards for Aseptic Surgery and the LASA Guiding Principles for Preparing for and Undertaking Aseptic surgery. Peri-operative care will be implemented following consultation with the NVS.

- We will also use rat models (normal and transgenic or knock-out) where required as they have genetic similarities to humans.

Why can't you use animals that are less sentient?

There is no suitable replacement or alternative to using the whole animal to study behavioural changes and related brain function. We need to use the whole animal in order to understand how changes in brain pathology/activity relate to behavioural/cognitive phenotypes observed during disease onset and progression. Many of the diseases (e.g. dementia) are age dependent and only appear following aging in the animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

- The development of new animal models that more truthfully mimic the symptoms seen in patients will allow us to understand the diseases better.

- Animals undergoing surgery that need to be single housed post-surgery will be single housed one week prior to the surgery in order to try and minimise the stress induced in the animals following surgery.

- Self-dosing of analgesia will be utilised in animals with analgesia given via drinking water, we have also implemented bespoke analgesia regimes depending on the strain of animals used and our experience of using them for surgical techniques. This has involved the administration of analgesia via drinking water prior to surgery.

- For behavioural testing we will utilise advanced video observation tools and novel computational analysis tools/software to facilitate a more sensitive and effective profiling of an animals behaviour.

- We have introduced standard operating procedures to regulate and standardise all methods and techniques used in our research to ensure consistency and high quality data with all researchers fully trained and assessed for competency. To try and minimise the harm and stress that the animals undergo they will be group-housed as much as possible during testing.

- All surgical procedures will be carried out using aseptic techniques and performed to the HO Minimum Standards for Aseptic Surgery and the LASA Guiding Principles for Preparing for and Undertaking Aseptic surgery. Peri-operative and anaesthetic care measures will be implemented following consultation with the NVS.

- Ageing rodents will be assessed and observed using scoring systems and humane end points established by other researchers with experience of ageing colonies and postoperative care typically includes the appropriate use of analgesics and fluid food rations to support recovery. Selection of behavioural testing will account for frailties in their state of health (for example reduced movement, sensory impairments like visual, auditory or olfactory).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

- We currently seek to implement a new Data Management System on the conduct of studies, their design and analysis tools, specifics required when working with collaborators from industry or academia and so on.

- At all stages of experimental design, we will follow ARRIVE 2019 guidelines with experimental blinding and randomisation in order to avoid any bias.

- We also use Design assist amongst other software tools for power calculation etc. We routinely use Matlab for writing new code for analysis scripts.

- Also, we follow the guidelines provided by commercial breeders (CRL: Guidebook on mouse and rat colony management) for housing and breeding of our colonies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continuously strive to apply the 3Rs to our work. In order to do this we will follow the literature, NC3Rs, CRACKIT and Norecopa.

Home Office

NON-TECHNICAL SUMMARY

141. Novel therapeutic targets for chronic kidney diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

diabetes, kidney complications in diabetes, chronic kidney disease, novel therapeutics

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim is to perform therapeutic proof-of-principle experiments to demonstrate that modulation of a certain cellular property called "alternative splicing" (either using chemical compounds or changing expression of certain genes) is a viable strategy for new treatments for chronic kidney diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Chronic kidney disease (CKD) is a major health problem worldwide. In US it is estimated that ~13% of the general population has CKD and in Europe the prevalence is higher at 16%. CKD is associated with increased risk of cardiovascular disease and mortality. It is therefore important to further our understanding regarding the molecular determinants of CKD and find new ways to use them for drug development. A large proportion of CKD is due to diabetic nephropathy, one of the complications of diabetes. In the Western word and UK, diabetic nephropathy is the leading cause of terminal renal disease and kidney replacement therapy (almost half of all patients with terminal disease) an immense burden on healthcare costs. It is estimated that 40-45% of patients with type I diabetes and 30% of patients with type II diabetes have nephropathy. Currently, there is no specific treatment for diabetic nephropathy – finding such treatments would be very useful, as slowing down kidney function degradation in diabetes would lessen the burden of caring for chronic renal patients.

What outputs do you think you will see at the end of this project?

The two parts of the project, as outlined in the background section, are at different stages of development, so the benefits will be explained separately:

1. Use of compounds that switch VEGF splicing

In previous work, both in vitro and in vivo, we have shown that the VEGFb isoform is beneficial to the kidney and it is lost in development of diabetic nephropathy. We have compelling evidence to move to next phase – test the hypothesis that compounds that are able to switch VEGF splicing will rescue diabetic nephropathy phenotype in proof-of-principle therapeutic experiments.

The data that will be collected is a thorough analysis of the kidney function to assess whether these compounds are indeed working to rescue the phenotype. This will be done in mouse models of type I and type II diabetes. Findings from this work will be made available to other scientists through publication in peer-reviewed journals and presentations at scientific conferences. In the last 5 years, we have published 12 papers related to alternative splicing in chronic kidney diseases and the potential to switch splicing therapeutically. The licence holder has been invited to present these findings at 10 international conferences and members of his group presented the data at 5 more conferences. We expect our scientific output to be similarly productive in the future.

The work proposed here is expected to be novel and to provide new concepts to the field of diabetes complications in two ways:

i. possibility of specific treatments to slow progression of kidney damage in diabetes – currently there is no specific treatment, only good glycaemic control is recommended

ii. proof that a new class of compounds – modulators of alternative splicing – may be used as new therapeutics in kidney diseases

2. The splice factor PTBP1 in polycystic kidney disease

This part of the project – while fitting in the big picture of contribution of alternative splicing to kidney diseases – is in a more early, basic science phase .

We will analyse comprehensively the kidneys and urine from the transgenic mice described in the background section. This data will be crucial to understand the mechanism on how PTBP1 mutation or deletion may affect the kidney and form the polycystic phenotype.
Findings from this work will be made available to other scientists through publication in peer-reviewed journals and presentations at scientific conferences – as described above .
The main novel contribution to the field by the work proposed in this project will be that a splice factor – PTBP1 – is able to promote a polycystic kidney phenotype, something that has not been reported before.

Who or what will benefit from these outputs, and how?

- **Molecular and splicing biologists**

Beside trying to develop novel therapeutics, this project will elucidate how several compounds signal to the splicing machinery resulting in a group of molecules that can be used as modulators of alternative splicing for further basic science studies. Furthermore, the project will link the science of splicing with functional intracellular and in vivo physiology readouts, i.e. link molecular pathways involving the genes proposed to be studied with the whole system functional biology of the kidney.

- **Diabetes and kidney researchers**

Splicing control is under-investigated in many diseases and this proposal uses as a model the diabetic nephropathy. Scientists working on diabetes and its kidney complications will become aware of a new area of the cell biology that they might not have thought of before – alternative splicing – and link various splicing molecules to their own research.
- **The Pharmaceutical industry**

may investigate the suitability of splicing control as a novel therapeutic strategy or use the link between splicing and diabetic nephropathy to test for potential effects of drugs.
- **Biochemists and chemists**

and those investigating how chemical moieties affect the interaction of molecules within the spliceome and/or spliceosome.
- **Clinicians and Patients**

One of the aim of the project is to develop drugs based on modulation of splicing that are able to conserve kidney function in diabetes and therefore may be investigated further by clinicians working in the field; in longer term we hope that new drugs to benefit patients with kidney diseases to result from this research
.

How will you look to maximise the outputs of this work?

To disseminate our research with colleagues, we have been, and will be in the future, regularly presenting our research at national and international conferences. These conferences will benefit us in terms of scientific discussion and new directions for the project, as well as forming collaborations within the diabetes research community.
To engage our research with diabetic/diabetic nephropathy patients, we will use diabetes focus groups already set up within our university. Research participants will be invited to seminars on our research held within the department, as well as specific presentations of our research in lay terms to Patient and Public Involvement (PPI) groups, which we hope to involve at least 10 patients. This will help us to evaluate the impact of our research to people affected by diabetes. We believe this is of particular importance as alternative splicing is a new and not well understood (in lay terms) area of research; therefore, more awareness of its importance is very much required. To achieve this goal, we will also attend our university's "Impactful Public Engagement with Research" course. Costs for a yearly seminar to a PPI group, including room hire and catering, as well as training costs are covered by our department.
Finally, the disseminate our research with the general public, we will use the Pint of Science platform, in which the postdoctoral researcher working on this project is a volunteer. This will aid us in communicating our research in lay terms, as well as opening up a wider scientific discussion of the importance of alternative splicing in diabetes.
Species and numbers of animals expected to be used

- Mice: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The function of the kidney is fully linked to the so-called "glomerular filtration barrier" - a dynamic structure that constantly has blood filtering through it. This is highlighted by the fact that in adult man 180 litres of fluid crosses this barrier every day. In the glomerulus there are 3 different cell types present, podocytes, glomerular endothelial cells and mesangial cells that are able to communicate with each other. The only way the glomerular filtration barrier can be assessed for how all these cells function together is with an animal model. The function of the glomerular filtration barrier in vivo is easy to assess by measuring the amount of protein excreted in urine and examining glomerular structure on histology slides. Where possible we will use our in vitro models but to fully investigate biological function we also need animal models. For instance, when investigating new drugs, we will do experiments extensively in vitro on cell lines derived in the kidney which will tell us, by measuring various cell properties, whether the compound is able act on their molecular target as intended and postulated or whether, for instance, it is particularly toxic. These experiments will be important in judging whether or not a compound is worth taking to the in vivo studies steps.
We are using adult mice as the simplest animal in which the kidney is very similar to tee human one.

Typically, what will be done to an animal used in your project?

Some of the mice from the so-called transgenic models will have a kidney condition that deteriorates in time because of the intrinsic genetic modification that they were exposed to. Some of the mice will receive a substance to make them diabetic or will be exposed to surgical procedures to artificially compromise kidney function.

Typically, in these experiments we will either have mice that are diabetic or mice that have renal dysfunction and we will administer these substances (compounds) either through injections or orally, in the drinking water. We will collect urine and blood and measure kidney function. At the end of the experiment we will collect the kidneys which will be analysed in detail through histological and electron microscopy techniques. We hope to obtain evidence that the administration of these substances slows the progression of kidney failure.

What are the expected impacts and/or adverse effects for the animals during your project?

The most common adverse effects are weight loss, polyphagia (excessive hunger), polydipsia (excessive thirst) or polyuria (excessive urination) from development of diabetes. Some toxicity signs may appear as described above, due to substances administration; however, we expect this to be minimum (less than 5% animals) since every single substance studied here will be either used before by us or assessed in pilot experiments as described above.

Careful monitoring of the animals is crucial for our studies, and we have strict criteria when animals are to be killed, depending on the status of their kidney function or metabolic disease. Animals will be inspected twice daily and the presence of signs of distress assessed. The design of our experiments will be such that we will minimise animal use but maximise data collection; often animals will act as their own controls, and repeated measurements minimises the number used. We will systematically monitor various signs and symptoms: weight, clinical signs post-anaesthesia (loss of coordination, abnormal breathing), swollen abdomen, gastrointestinal problems (diarrhoea), hunched posture, piloerection, restlessness, less mobile and alert, isolated, vocalisation, self-mutilation. Appropriate action will be taken e.g. monitor carefully/ consider analgesics, consult NACWO and NVS, consider termination.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

mild - 20% moderate

- 80%

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The function of the kidney is fully linked to the so-called “glomerular filtration barrier” - a dynamic structure that constantly has blood filtering through it. This is highlighted by the fact that in adult man 180 litres of fluid crosses this barrier every day. In the glomerulus there are 3 different cell types present, podocytes, glomerular endothelial cells and mesangial cells that are able to communicate with each other. The only way the glomerular filtration barrier can be assessed for how all these cells function together is with an animal model. The function of the glomerular filtration barrier in vivo is easy to assess by measuring the amount of protein excreted in urine and examining glomerular structure on histology slides. Where possible we will use our in vitro models but to fully investigate biological function we also need animal models.

Which non-animal alternatives did you consider for use in this project?

We regularly use in vitro cell cultures with various kidney cells to test our hypothesis.
For instance, when investigating new drugs, we will do experiments extensively in vitro on cell lines derived in the kidney which will tell us, by measuring various cell properties, whether the compound is able act on their molecular target as intended and postulated or whether, for instance, it is particularly toxic. These experiments will be important in judging whether or not a compound is worth taking to the in vivo studies steps.

Why were they not suitable?

We can assess various properties of kidneys cells in culture, however, we cannot assess the kidney function as a whole organ just by culturing cells.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Mice numbers were estimated depending on how many experimental animals will be needed. Statistical power calculations using data from diabetes experiments routinely performed by the
applicant show that n=10 experimental and n=10 control mice are needed to see a significant difference ($p>0.05$) in albuminuria with a power value of 0.80 (>80%). More specifically, we have used raw data from our previous experiments in db/db diabetic mice in which albuminuria was compared with their lean controls over time and was shown a significant difference between the two groups. From these data we calculated the averages and standard deviations for each time-point. Because the two populations have different standard deviations, for power calculation we have used an online tool which is appropriate for comparing two populations with unequal variance:
http://epitools.ausvet.com.au/content.php?page=2Means2
Using these calculations we have found the most appropriate group size to be formed of 10 mice. These calculations were done together with a statistician.
Maintenance of transgenic colonies – the average number of mice in a colony to be able to provide at least 3 breeding pairs and enough litters to supply the experimental cohorts is estimated at 10.
Experimental numbers of mice were estimated based on the number of compounds we plan to use under this licence, over 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will minimise the number of animals necessary by following the principles of reduction by thorough statistical analysis as taught on module 5 of the Home Office licensing course.
Many of our studies require cross breeding of animals with different genetic mutations. To limit numbers, we will set up colonies in which we optimize the number of offspring that have the correct genotype. This will mean that fewer mice have to be sacrificed prior to being part of an experimental study.

If giving the mouse a substance and the response to different doses needs to be ascertained, we will initially perform pilot studies to ascertain the correct magnitude of drug required to induce an effect and then go on to a formal hypothesis driven experiment. If using novel drugs, we will include a prior stage to ascertain safety, quality of manufacture and suitability (e.g. vehicle and pH) of the drug. This will be performed by an extensive literature review of the drug (or associated drugs) and their safety profile. We will then perform a pilot experiment in a single mouse using a low dose of the medication and will closely monitor the mouse for side-effects before using it on greater numbers. We will consult an expert statistician before embarking on our studies to ensure the minimal number of animals to derive meaningful results is studied. To this respect I have a continuous collaboration with a statistician from our university
.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Mice breeding and colony maintenance will be done in accordance with the ASRU Efficient Breeding of Genetically Altered Animals Assessment Framework. Mice numbers will be kept to a minimum by using crossing designs that result in minimal animal numbers (e.g. we will use homozygous breeding pairs when possible), demand will be assessed before breeding and crossing, colonies will only be maintained while there is an experimental plan and funding allocated.
We perform breeding calculations before we plan our experiments and only produce the numbers of animals that we need.
We have a database that tracks all this information and also gives me historical data. It is very easy to see how many animals there are in each colony, how frequently they are producing litters and the size of the litters. It is easy to see when the pairs need to be replaced.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We are using several mouse models:

- a transgenic mouse in which a certain protein is missing (knocked out) from the kidney; this is the only way to understand the role of this protein in the kidney and is modelling what happens in the kidneys in human patients when this protein is missing

- transgenic mouse models of diabetes; the ones we selected are the best to study diabetic kidney disease, as in many other mouse models of kidney disease the kidney is not damaged; therefore to be able to study the kidney function in diabetes these kidney-sensitive mice will be kept the shortest time possible

- chemically-induced diabetes model – a chemical named streptozotocin is injected to destroy some of the pancreatic cells; this will provoke the appearance of diabetic disease similar to what is seen in humans; fairly quickly in this model, after a couple of weeks, signs of diabetic disease of the kidney also appear, and therefore we can perform our experiment sin the shortest of time possible

The methods we are using are the minimal needed to be able to analyse the kidney function - urine collection, blood sampling and blood pressure measurements; we will also test substances that we believe are able to preserve kidney function but this will be done only after pilot studies in which we test toxicity and make sure the mice will not suffer
.

Why can’t you use animals that are less sentient?

To be able to study the complexity of the kidney as an organ we need to do this in mammals like mice. Embryonic forms cannot be used as kidneys are in development
.

Models less sentient, like zebrafish, do not have a kidney similar to the human kidney

.
Experiments are needed for a longer time period and need to mimic diabetic disease, so cannot be done in terminally anaesthetised animals
.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I constantly think on how to refine the procedures we are using. We review with my team all the procedures before the start of each experiment and discuss whether there are better ways to do them; make sure that animals are well accommodated (e.g. always leave at least 7 days from delivery to the start of the experiment); make sure there is proper monitoring (e.g. once the mice become diabetic and show clinical symptoms like excessive urination we assess their welfare daily and sometimes twice daily).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Several of the NC3R guidelines: https://www.nc3rs.org.uk/guidelines

https://www.nc3rs.org.uk/3rs-resources/ https://www.nc3rs.org.uk/3rs-

toxicology-and-regulatory-sciences https://www.nc3rs.org.uk/3rs-

resources/blood-sampling https://www.nc3rs.org.uk/how-to-pick-up-a

mouse

Other: http://www.procedureswithcare.org.uk

LASA (Laboratory Animal Science Association) guiding principles:

http://www.lasa.co.uk/PDF/AWERB_Guiding_Principles_2015_final.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I am a registered user on the NC3R website (National Centre for the Replacement, Refinement and Reduction of Animals in Research); as such I receive regular updates and newsletters with new advances in the field.

I am constantly in touch with the Named Animal Care and Welfare Officers as well as Named Veterinarian Surgeon at our institutions and we discuss various novelties in animal care and techniques.

Home Office

NON-TECHNICAL SUMMARY

142. Ocular gene therapy

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Rats	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The overall aim of this project is to develop new methods to treat various blinding eye diseases by using gene therapy
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The eye diseases we are targeting are currently either poorly treated or there are no effective treatments for them at all. Although many of these diseases are relatively rare, their impact on the individual patient and the community is substantial.
We are already performing clinical trials of gene therapy for retinal dystrophy and we will initiate several more clinical trials on inherited and acquired disease in the coming years based on the work proposed here. We hope that accomplishing these clinical milestones will be an essential first step in the development of a treatment for these untreatable, blinding diseases.

What outputs do you think you will see at the end of this project?

The overall aim of this project is to develop new methods to treat various blinding eye diseases by using gene therapy. Potential outputs include novel gene therapy vectors for use in clinical trials. Findings will also be documented in Intellectual Property (IP) filings as well as peer-reviewed research publications and conference presentations.

Who or what will benefit from these outputs, and how?

The eye diseases we are targeting are currently either poorly treated or there are no effective treatments for them at all. Although many of these diseases are relatively rare, their impact on the individual patient and the community is substantial.
Preclinical proof of concept and toxicology data acquired from the proposed studies, will lead to successful candidates for treating retinal degenerations entering clinical phase development. This will include Good Manufacturing Practice (GMP) material manufacturing and Phase I/II clinical trial initiation (as managed by other departments of our company).

How will you look to maximise the outputs of this work?

Where needed, we will seek additional academic or third party collaborations to expedite the development of these novel gene therapy vectors to treat forms of blindness.

Species and numbers of animals expected to be used

- Mice: 5000
- Rats: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The visual cycle is a complex process that occurs in the photoreceptor cells and as such in vivo experimental platforms are much more informative that in vitro ones. Animal models often lack function of the orthologue gene that occurs in mutated forms in patients and as such are excellent models of disease for proof of concept studies.

Depending on the underlying mutation, these animal models develop retinal regeneration at different ages and treatment within certain periods of development can help establish the window of opportunity for treatment in patients.

Typically, what will be done to an animal used in your project?

Once an animal reached the appropriate age for intervention based on the underlying cause of disease, an intraocular injection with an investigational gene therapy vector will be performed.
In-life non-invasive assessment of vision will be performed at various time-points. Duration of the in-life phase depends on the severity of the retinal degeneration and/or efficacy of the gene therapy vector. It can extend to maximum 14 months. Following necropsy, all relevant tissues (i.e. eyes and major organs) will be assessed for efficacy of the tested drug.

What are the expected impacts and/or adverse effects for the animals during your project?

All animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours following intraocular procedures.
Uncommonly animals that fail to do so or exhibit signs of:

- pain,

- distress/significant ill health (i.e. general signs of ill health such as hunched appearance, piloerection, lethargy etc.)

Animal exhibiting these signs be killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers
Any animal not fully recovered from the surgical procedure within 24 hrs (eating, drinking and return to normal behaviour) will be humanely killed.
Additionally, any animal exhibiting a maximum 15% loss of weight at any point, will also be humanely killed.
Specific adverse effects from intraocular administration include ocular infection. This is rare but in the case it occurs, treatment advice from the NVS will be sought. If the animal does not respond to the treatment within 48hrs, it will be killed by Schedule 1.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild: 5000 mice (100%), 100 rats (100%)

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Although therapeutic vectors are tested in cultured cells prior to use in animals, treatment efficacy can only be proven in animals, as the diseases we aim to treat (like retinal degeneration) are complex disorders, involving interactions between multiple cell types throughout the retina. These interactions cannot be tested in cultured cells.

Which non-animal alternatives did you consider for use in this project?

In vitro culture systems and/or computer modelling

Why were they not suitable?

Current knowledge and techniques are insufficient to model all these interactions, either in a culture system or using computers, well enough to reliably predict treatment outcomes. However, we are using cultures of 3D retinal organoids that harbour the same mutations as the animal models of interest in the human orthologues. Currently, no functional assessments can be performed using the retinal organoids
(this is something that in vivo work is essential for) and they are used to assess expression of transgenes and protein localisation in human tissue.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This estimate is based over 5 years based on:

- Projected number of projects (8 projects, some overlapping)
  Projected duration of each project (12-18 months depending on model)
- Projected needs for in vivo proof of concept (efficacy) and toxicology

-

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Power calculations, employment of SOPs and design based on experience from previous preclinical programs that we have successfully progressed to the clinical stage as well as designs using NC3R's EDA software.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies will be employed where appropriate (Protocol 2 and 3) and breeding advice will be given by the staff of the establishment where breeding and maintenance of animal lines will take place.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The models employed will have certain forms of retinal degeneration. The intervention will mirror the safest surgical approach that is aimed for patients and the majority of assessments will include minimally invasive tests while the animals are alive, and as such collecting as much data as possible from each animal.
The testing of novel therapeutics aims to improve or halt the retinal degeneration observed in these models.

Why can't you use animals that are less sentient?

Live animals need to be used so that vision can be assessed using both electrophysiology and behavioural assessments. Additionally, mice are the lower vertebrate group with a fully post-mitotic retina, and they will answer all scientific questions as adequately as larger animals.
Animal of lower order (such as amphibians or fish) lack the anatomical relevance and adequate common features in terms of retinal function in order for them to act as useful models for these gene therapy research studies.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have developed improved gene therapy vector production techniques, which give far better vector purity than previously established techniques and thus will further decrease the (already low) risk of inflammation post-administration.
Regular observations will be performed as required by all protocols used to ensure the welfare of the animals taking part in this project. In addition, we are looking to develop novel agents that will allow for treatment through optimised routes of delivery (i.e. intravitreal instead of subretinal delivery).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The ASPA, LASA and ARRIVE guidelines will be followed together with current developments in the field in order to refine the experiments using most up-to date discoveries in gene therapy.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

Everybody involved in this project will be in close communication with the establishment, the NACWO, NVS and the regional NC3R's manager throughout the duration of the project making sure any advances in the 3Rs are implemented. Advice will be sought from Regional 3R’s Manager as required

Home Office

NON-TECHNICAL SUMMARY

143. Oxidative stress-related mechanisms of liver fibrosis.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Liver fibrosis, oxidative stress, Nrf2

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We will use mice where the Nrf2 pathway has been genetically modified to examine cell type-specific redox signalling pathways that are regulated by Nrf2 in the context of liver fibrosis. Specifically, we wish to evaluate whether activation of Nrf2 in hepatic stellate cells or in macrophages allows either liver fibrogenesis to be arrested, or established fibrosis to be resolved.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Fibrosis of the liver is a global healthcare issue, with obesity-driven NAFLD (i.e., non-alcoholic fatty liver disease) reaching epidemic proportions in the Western world. A substantial number of affected patients will progress to liver cirrhosis and thus risk developing hepatocellular carcinoma. Moreover, there is currently no effective treatment for liver fibrosis other than transplantation, and this is a limited option: new therapeutic strategies for liver fibrosis are therefore urgently required.

What outputs do you think you will see at the end of this project?

We believe this project will provide new information on the development and resolution of liver fibrosis. We also expect to be able to publish several papers from the work in this project.

Who or what will benefit from these outputs, and how?

Worldwide there is an increase in obesity- and Type 2 diabetes-related liver disease that can lead to fibrosis. There is currently no therapeutic treatment other than transplantation for liver fibrosis and there is a shortage of transplant donors. Through this research we hope to translate our findings about how activation of NRF2 arrests/reverses liver fibrosis into addressing an unmet clinical need, and to inform future clinical trials.

In addition, our collaborators and other research groups within the same/similar fields will benefit from our work on NRF2 through the dissemination of new knowledge in the short-term. In the longer-term we hope that these outputs will benefit translational studies and eventually patients suffering from liver fibrosis. At the moment there are no non-invasive treatment for liver fibrosis and we hope that our research lays the foundations for the development of new pharmaceutical agents within this field and /or the repurposing of current drugs.

How will you look to maximise the outputs of this work?

We will maximise the outputs of this work through collaborations with other institutes and disseminate new knowledge through publication of peer reviewed papers.

Species and numbers of animals expected to be used

- Mice: 6000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will be using mice as they are able to be genetically altered which will allow us to spatially and temporally

activate the genes and cells of interest. The human diseases, e.g. liver fibrosis, we are studying most often occur in adults. We are using adult mice as these reflect the disease in humans.
Typically, what will be done to an animal used in your project?

Animals used in this project will be injected i.e. not more than twice per week with known agents causing liver fibrosis e.g. halogenated hydrocarbons over a period of 6 weeks. Some of the animals will also be treated with agents expected to induce changes in expression of genetic alterations, starting before this period and/or extending into it. All animals will be killed and tissues taken for detailed laboratory analysis.
What are the expected impacts and/or adverse effects for the animals during your project?

The expected impacts and/or adverse effects for the animals are acute liver injury or liver fibrosis. This is not usually associated with pain in humans and therefore unlikely in mice. (Scholten et al (2015) Laboratory Animals 49(S1): 4).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severities would be mild to moderate with about 50% in in each category **What will**

happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Liver fibrosis is a three-dimensional process involving cells of different types. It cannot yet be modelled satisfactorily in non-animal systems.
Which non-animal alternatives did you consider for use in this project?

We considered using cell/tissue cultures and advanced computer modelling techniques. We are using human derived cells for relevant aspects of the project as an alternative to animal use.
Why were they not suitable?

Cells do not reflect what happens in the whole living body and so can only be used to answer certain scientific questions. Animal models help to ensure the effectiveness and safety of new treatments by providing information on responses within the whole organism.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Initial groups of 10-12 mice will be generated to assess the potential magnitude of the response to the induction of chronic liver fibrosis. Based on these initial data the number of mice will be amended appropriately in order to gain sufficient statistical power for hypothesis-testing experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We researched literature precedent, used online tools such as G*Power, and will follow the guidelines set out in the ARRIVE and PREPARE Guidelines.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To optimise the number of animals used in this project we will breed our various genetically modified lines as efficiently as possible. We will also run a small pilot study and use the results to help to determine the lowest numbers required to give statistically significant results.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mice as the animal models for this project which reflect liver fibrosis in humans. The mouse genome is very similar to that of the human genome making mouse genetic research particularly useful for the study of human diseases such as liver fibrosis. The methods used will be i.p. (Carbon tetrachloride, CCl4) to initiate liver fibrosis and gavage (TBE-31/tamoxifen) to treat liver fibrosis/induce
Cre in a tissue specific manner respectively. There may be pain/discomfort associated with the initial CCl4 injection, but analgesics will be used to mitigate this, as advised by the Named Veterinary Surgeon. Liver fibrosis itself is not usually associated with pain in humans and this is therefore unlikely in mice. These methods will only cause transient pain/discomfort to the animals.

Why can't you use animals that are less sentient?

Liver fibrosis occurs most often in adults. Therefore it is important that our animal model reflects that life stage. Lower vertebrates and invertebrates have differences in the microenvironment which may contribute to species-specific responses to injury. Liver fibrosis is a progressive disease and our study aims are to resolve the fibrosis.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimise the welfare costs for the animals we will monitor and weigh the mice throughout their treatment time.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The published best practice guidelines we will follow will be the ARRIVE guidelines at https://www.nc3rs.org.uk/arrive-guidelines and the PREPARE guidelines at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5862319/

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

We will stay informed about advances in the 3Rs through frequently checking the website and resources offered at https://www.nc3rs.org.uk/3rs. We will also be in consultation with our unit NACWO.

Home Office

NON-TECHNICAL SUMMARY

144. Papillomavirus Transmission, Infection and Lesion Formation

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Papillomaviruses, Cancer, Infectious Disease, Virus Transmission, Viral Immunity, Viral Immunity

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Rabbits	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To establish how papillomaviruses are transmitted, how lesions form and are maintained, and to find new ways to prevent transmission and treat and treat clinical disease .

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Papillomaviruses (PVs) have been discovered in a wide array of animals. More than 300 PVs have been identified, including over 200 human papillomavirus (HPV) types. HPVs infect humans and cause epithelial lesions with different clinical outcomes, ranging from non-cancerous hyper-proliferative lesions such as warts, to subclinical (not apparent) lesions that can in some instances progress to high-grade neoplasia and cancer. HPV infection is responsible for approximately 6.1% of all human cancers including cervical, head and neck, anal and conjunctival cancers, however 90% of HPV infections are cleared by the host immune response. HPV vaccines have been proven to be effective and are expected to make great impacts on HPV-related disease, but have some limitations:

a) They are only effective for individuals who have never been infected with vaccine type HPVs (i.e. they are prophylactic rather than therapeutic).

b) Because of their cost, these vaccines are not widely available in developing countries where resources are limited.

c) Vaccine hesitancy is restricting implementation even in developed countries with vaccine programs.

Therefore, there is ongoing need for the development of antiviral agents to treat existing HPV infections, and an efficient strategy for the management and control of HPV infections through screening, including cervical screening, which is currently being modified to incorporate HPV DNA testing .

As HPVs are host-specific and cannot infect other animals, and there is no precise animal model for HPV infection. However, the general strategy of all PVs to accomplish their various life cycle stages in the skin are similar, with different papillomaviruses having similar molecular functions for their gene products. Although many aspects of the infection route are not fully worked out i) PVs target the skin, which consists of multiple cell layers; ii) after PVs have infected cells in the lower (basal) layer of the skin, infected cells are maintained for years to decades, modulating proliferation and commitment to differentiation of the skin cells; iii) Once the infected cells leave the basal skin layer, the productive life cycle is triggered and new viruses are produced; iv) PV is shed inside dead cells from the surface of infected skin, and transmitted via direct or indirect contact; v) PV infection is controlled by the host immune system, which modulates viral gene expression.

Given this common strategy of PV infection, we aim to use animal models to elucidate how PVs are transmitted, and how new lesions form and are maintained, in order to develop new insight into PV transmission mechanisms, and the development of anti-papillomavirus agents/treatments and infection control strategies. These aims can be achieved using models that allow us to investigate all aspects of the virus life cycle (transmission, infection, lesion formation, and the production of new virus) in the presence of an intact host immune system.

What outputs do you think you will see at the end of this project?

During this project, we aim to better understand:

1. How mouse PV is transmitted and infects new sites, particularly how virus preparation (purified virus, cell-free virus, and virus in exfoliated dead cells (squames)) affects virus survival and infectivity. We will evaluate the efficacy of disinfectants currently used in clinical settings on virus infectivity. Together with a parallel analysis using human PV in tissue culture models, we will examine how papillomavirus transmission can be controlled and prevented. We expect to achieve this goal within 3 years.

2. The early stages of lesion formation following infection. We want to define the molecular pathways that are modulated by the virus to maintain infection in the basal (lower) layer of skin cells, and how virus genes do this (achievable in 3 years). We also want to develop ways of manipulating these pathways to inhibit the maintenance of infection in the basal keratinocyte (skin cell) layer, leading to the development of anti-papillomavirus therapy/agents. This will depend on the progress of a parallel study using keratinocyte tissue culture models to investigate the function of virus protein function on keratinocyte proliferation and differentiation. However, we expect to have a rudimentary understanding of this within the 5-year period.

3. How mouse PV gene expression patterns in mice vary at the different body sites in mice (achievable in 3 years). Our longer-term aim is to understand how virus gene expression patterns contribute to the difference in the behaviour of the infected lesion (phenotype), and to relate such differences to expression seen in clinical samples infected by HPV. We expect to have a basic understanding of this within the 5-year period.

4. The molecular process that drives lesion regression. This is a more substantial goal than those described above, but during these 5 years we aim to look at the lymphocytes (small white blood cells) and the host's immune response in the lesions that regress.

We estimate that four major publications will come from this proposed work, the first relating to ‘Papillomavirus transmission, lesion formation and disinfection’, a second relating to ‘The molecular mechanisms of early stage papillomavirus lesion formation’, a third relating to 'The process of lesion regression of papillomavirus infection; the implications of the host immune system', and a fourth relating to 'Tissue culture models of the cervical transformational zone, and the dysregulation of viral gene'.

Who or what will benefit from these outputs, and how?

The results will benefit the scientific community, by providing a comprehensive understanding of how PVs are transmitted and form lesions, and how infection is controlled by host immunity. The impact of outputs from the first objective (viral transmission) will provide a new insight into how HPV transmission can be controlled and prevented, which will directly affect clinical practice. In the longer term, the outputs from the second and third objectives will provide identify key cellular pathways that PVs regulate to form and maintain lesions at specific sites of infection. Those interested in the development of anti-HPV treatments/agents will also benefit from this work.

How will you look to maximise the outputs of this work?

The scientific results will be disseminated globally primarily through publications as well as preparation of review articles, book chapters, and lectures at scientific and other meetings.

Species and numbers of animals expected to be used

- Mice: 3710
- Rabbits: 96

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

There is no experimental model to investigate all stages of PV infection (infection, lesion formation, virus production, and virus regression) without the use of animal models. Only animal models will allow us to investigate all aspects of these stages. Each PV is highly host-specific and can infect only their natural host. However, all PVs are considered to share fundamental strategies in their life cycle. Thus, we can understand how the human PV transmits, infects and forms its lesion by investigating animal PVs. Using the mouse PV model, we can also investigate virus transmission and lesion formation using various genetically modified mouse strains. With the rabbit PV model, we can specifically investigate how the host immune system responds to PV infection and resolves it.
Some PVs, including HPV, cannot be propagated using a tissue culture model. Transplanting infected cells into mice is currently the only way that we can propagate infectious PVs for use in other experiments.

Typically, what will be done to an animal used in your project?

Small areas of skin will be gently scarified before infectious material is applied. A wart-like lesion will grow at the inoculated site and the development, and in some cases regression, of the lesion will be monitored over a period of up to 6 months.
After virus infection, animals will receive reagents or treatment (e.g. cryotherapy) that may affect the process of lesion formation/persistence. The number of procedures that mice and rabbits will be subject to will be kept to a minimum to limit discomfort, while allowing the generation of scientifically useful data. A set of procedures will be repeated no more than three times per animal, with intervals between treatments that allow for full healing (e.g. following mechanical abrasion). Ten percent of animals may receive both reagents and treatment, the rest will receive one of the two, or neither. Animals will be continually monitored for adverse effects post-treatment, and if necessary, the intervals between treatments will be adjusted or the treatment will be suspended.
Similarly, rabbits will be inspected regularly and, in some cases, biopsies (up to three) and blood samples (up to two) may be collected.
For infectious papillomavirus production, tissues/cells will be transplanted into mice under the coating covering each kidney by surgical procedure. Transplanted tissue/cells will be collected after three months, after the animals have been killed.
Genetically modified mouse strains which are not commercially available will be maintained/bred under this project, and procedures required for this will be carried out. None of the genetically altered mice are expected to develop any adverse effects.

What are the expected impacts and/or adverse effects for the animals during your project?

Proliferative lesions will develop at the site of infection (warts). In mice, the lesions are self-limiting and localised, and will not affect the general health and welfare of the animals. Animals will be killed by a humane method before they show moderate clinical signs, such as large lesions or secondary lesions that interfere with the normal processes of feeding and movement within the cage. In rabbits, the lesion naturally disappears within 4 months. In mice, the transplanted tissue/cells will not cause any clinical signs.
Animals will be closely monitored during and after each procedure, and the animal will be treated if adverse clinical signs (such as weight loss, lack of normal movement or other abnormal behaviour) are seen. If there is no response to treatment, the animal will be killed by a humane method. Bodyweight will be recorded when the experiment starts and will be monitored regularly and compared with age and ‘free feeding' matched control animals control animals.

Although we expect some transient soreness at the scarification site immediately after infection, the non-cancerous lesions that develop as a result of infection are not expected to be painful for the animal. Painkillers will be provided if required.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of mice (95.4%) will experience procedures of only mild severity, with the remaining mice (4.6%) undergoing procedures of moderate severity.
Only mild severity procedures will be used with Rabbits.

What will happen to animals at the end of this project?

- Kept alive
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We can investigate all the stages of PV infection from transmission/infection, lesion formation, and lesion regression, to reactivation at particular epithelial sites, only by the use of animal models. Importantly, we can only investigate the role of the immune system in PV lesion regression using the rabbit PV system. By using these animal models we can produce a more complete picture of PV/host interactions interactions.

Which non-animal alternatives did you consider for use in this project?

We already make extensive use of tissue culture and organotypic raft culture (which allows the formation of skin tissues in the lab), to study the papillomavirus life cycle. We also employ a culture system to study infection and lesion formation in patient biopsy material. Additionally, we compare the results from animal experiments with clinical observation of patient material. Such comparative analysis allows us to develop our understanding of how papillomaviruses interacts with skin (epithelial) cells.

Why were they not suitable?

The raft model and analysis of clinical specimens are useful; however, we need to use animal models to address particular questions concerning early lesion formation, regression and the associated immune responses. In addition, some human papillomaviruses can only currently be grown outside of their human host, in animal models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Production and maintenance of genetically altered mouse colonies (2 strains concurrently) is estimated to require 2000 mice over 5 years. The standard protocols for genetically altered mice (protocols 1 - 5) may require 410 mice over 5 years, as we expect to need two strains to make the embryo stock and/or to re-establish the strain from the sperm or embryo stock.
The PV infection protocol (protocol 6) is estimated to require a maximum of 1000 mice (24 mice per experiment, for 50 experiments) over 5 years. The production of 'infectious PV protocol' (protocol 8) is estimated to require 100 mice (4 mice per PV stock, for 25 PV types) over 5 years.
The rabbit PV infection protocol (protocol 7) is estimated to require 100 rabbits (8 rabbits per experiment, for 12 experiments) over 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Our experiments will be exploratory, and primarily observational or qualitative. They will address different features of disease pathology and viral gene expression, such as how the host immune system controls infection, how PV changes the behaviour of infected cells to form lesions, as well as how the site of infection affects PV behaviour.
Substances which are expected to modulate virus function are commercially available, and routes of administration, dosage volumes, frequencies and durations will be based on established protocols from published literature. We will use the NC3Rs experimental design assistant tool and the PREPARE guidelines and submit a protocol amendment along with justification if our analytical requirements change in future.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We are already involved in the analysis of virus expression and virus protein function in clinical specimens and skin (epithelial) tissue culture models, and we are using these models to develop our hypothesis of how PVs interact with the skin cells (keratinocytes) that they infect. Before conducting any animal experiments, we will make extensive use of tissue culture and organotypic raft culture (a system that permits 3D modelling of virus infection in lab recreated skin tissues), to study the biology of PVs in vitro. Experiments which specifically require the use of animal models will be conducted once this has been done.
We will keep animal strains that are not commercially available as embryo stocks, and will breed and maintain living colonies only when necessary during the project in order in order to minimise the use of animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Previously, the most appropriate animal model for the study of PV infections was the rabbit, and we have used the cottontail rabbit (CRPV) and rabbit oral (ROPV) papillomavirus systems in previous studies. These models are difficult to use, and we have found that rabbits may suffer weight loss upon treatment with immunosuppressant drugs. The identification of a mouse PV has taken time to achieve, but recently one has been described in the literature and is reported to produce typical papillomas (warts) in genetically modified immunodeficient mice. As a result, we can now assign the majority of our studies to the mouse PV model. However, the rabbit PV models are still useful, and essential when studying how the host immune system responds to PV during the natural course of infection. Other papillomavirus models are in larger animals (cows, dogs) or unconventional laboratory animals (e.g. the multimammate rat).
Papillomavirus lesion formation requires scarification of the skin to allow access of the virus to the lower (basal) epithelial layers. Scarification is minimised to limit bleeding and is performed under anaesthetic. The process of lesion formation causes no pain to the animal, and will routinely be carried out on the tail.

Why can't you use animals that are less sentient?

Each PV can only infect and form lesions in its natural host, and as a result we need to use mice to study the mouse PV, and rabbits for the rabbit PV. We are using animals of the lowest sentience that are susceptible to PV infection.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Working closely with the biomedical services staff, we will monitor animals on a daily basis. Animals must be allowed to acclimatise to new surroundings and personnel before any procedures are performed, to minimise stress and safeguard animal welfare and research results. Personnel will be trained to handle mice and rabbits correctly. Especially during and after procedures, animals will be monitored closely for adverse clinical signs, and the experiment/procedure will be terminated if necessary, to stop suffering. It is expected that animals will endure some discomfort associated with the surgical procedure. This will be minimised by use of good surgical technique, and pre-emptive and post-operative analgesia will be administered.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the Laboratory Animal Science Association (LASA), PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We subscribe to the Tech3Rs newsletter published by NC3Rs, and will keep up-to-date with our department's circulated 3Rs publications and website.

Home Office

NON-TECHNICAL SUMMARY

145. Pharmacokinetics of Pharmaceuticals

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Pharmacokinetics, Medicines, Preclinical

Animal types	Life stages
Mice	juvenile, adult, pregnant
Rats	juvenile, adult, pregnant
Rabbits	adult
Guinea pigs	juvenile, adult
Cynomolgus macaques	juvenile, adult
Beagles	adult, juvenile

| Animal types | Life stages |

Minipigs	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this work is to conduct relevant studies during the development of new medicines, to assess how much and how quickly the body absorbs the test medicine, and then how much and in what ways the medicine is changed within the body and then excreted. **A retrospective assessment of these aims will be due by 09 April 2026**

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The work in this project will allow drug companies to make decisions on which possible new medicines are most likely to be to worth spending more time and animal use in developing, and also then providing enough information for the government regulators to allow the safe administration of the materials to people. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework.

What outputs do you think you will see at the end of this project?

Data collected will be information on the absorption of various potential new medicines, as well as how animals change and excrete these materials. Outputs will include simple measures like the amount of the medicine which is in the blood, urine or faeces, at various times after being given the medicine. These results can then be compared to the known or expected situation in people.
The data will be collected to the quality standards expected by government regulators in the UK, Europe and elsewhere, who will make decisions on whether these materials can be safely marketed and used in society. Improved methods of conduct of specific data collection processes may be developed during the course of the project.

Who or what will benefit from these outputs, and how?

Our clients, typically commercial drug companies, will benefit from the provision of high quality data. This will to

help them in their work to develop new and better medicines, to discontinue development of inappropriate medicines or to understand and manage the risks of new medicines given to people.
Work on this project may also provide data to inform ongoing human clinical trials.
Enabling development of successful medicines will benefit society through diagnosis, treatment or prevention of disease.
The wider scientific community may benefit from publication of refined approaches to animal use.

How will you look to maximise the outputs of this work?

Our organisation has colleagues who also have experience of such work in different parts of the world. Collaborations and information exchange with others within the organisation, helps to identify and spread information on successful and unsuccessful approaches.
The licensee and colleagues have had on-going collaborations with NC3Rs on various aspects of the conduct of studies, and associated housing and husbandry methods for the animals, over many years.
The licensee and colleagues seek to disseminate information through presenting outputs at scientific conferences and contributing to publications in the scientific literature where relevant. **Species and numbers of animals expected to be used**

- Beagles: 250
- Minipigs: 150
- Pigs: No answer provided
- Cynomolgus macaques: 250
- Mice: 5000
- Rats: 10000
- Rabbits: 100
- Guinea pigs: 50

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Many scientific studies have been conducted to demonstrate that the types of animals to be used in the project will provide results which reflect the likely outcomes for people. Where this is uncertain, comparison between results in different species may be conducted, to identify the most relevant for predictions of outcomes in people. The way in which each new medicine is absorbed, changed and removed by people may be known, or predicted from other information, or studies may be conducted to find this out. The animal type(s) to be used will be chosen based on this understanding, or to find this out. The stage of life of the animals to be tested reflects the age/stage of life of people who would receive the medicines.
Another big advantage of using the listed animal types is that these animal types may be recommended by specific guidelines on how to do this work, and the results of tests are known to be acceptable to the government agencies responsible for authorising use of the medicines in human volunteers and patients. Development of new medicines cannot currently be achieved without this approval by government agencies in the UK, elsewhere in Europe and in other parts of the world.

Typically, what will be done to an animal used in your project?

Animals will be given a potential new medicine by the same method that people would be exposed to them - most commonly by mouth, but may be by other routes, including by injection and application to the skin. The

medicines are most commonly given once only, and samples such as blood samples are taken to assess how much of the medicine has been absorbed by the body. Similarly, samples of urine and faeces and expired air may be collected to see how much and in what way the body excretes the medicine. Collecting urine and faeces and expired air requires keeping the animals, normally singly, in a small cage which allows the urine and faeces to fall through a grid, typically for about a week.
Some animals will have surgery conducted under anaesthesia, and with use of pain relief, to allow collection of bile and/or blood from animals after they have been dosed and housed as described above.
Animals may be humanely killed after the collection period, and tissues may be taken from the animals post mortem (after death), and analysed. Often, animals are kept, and after checks by a vet to confirm that no lasting harms have been caused, they may be used on repeated occasions.
Some animal use involves only taking blood samples, to conduct scientific studies using the blood only.

What are the expected impacts and/or adverse effects for the animals during your project?

The process of dosing animals, taking samples and confining for collection of urine/faeces/expired air can cause a degree of discomfort during conduct, but not expected to be long-lasting. The dose of test medicines used is not normally expected to cause any significant harm for the animals. Surgery has the potential to cause pain or discomfort, but this is generally prevented or minimised by use of appropriate anaesthetics drugs and pain relief, under veterinary control.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The harms described above are expected to fall very largely within the mild category, as most studies involve giving a non-harmful material and taking a series of blood samples. Where surgery is performed, or animals are single housed for a period of days for collection of urine and faeces, this would be noted as moderate severity, and may involve about a tenth to a quarter of the total number of animals. Severe outcomes are not anticipated; if seen in individual animals, these would be reported to Home Office.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Rehomed

A retrospective assessment of these predicted harms will be due by 09 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Non-animal methods are routinely used in some aspects of the development programme for new human medicines, but they are currently not able to sufficiently predict effects on whole body systems or to provide information on how much of a medicine is absorbed, changed and excreted. This information is essential, to

confirm that possible new medicines should be developed, and to protect human volunteers and patients who may then take the medicines.

Which non-animal alternatives did you consider for use in this project?

The organisation does conduct various non-animal tests as part of the development programme for new medicines, but as noted above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes. Some studies are conducted using only blood samples taken from animals; taking the blood samples for this work is also included in the project.

Why were they not suitable?

There currently remains general scientific agreement, and agreement of government regulators, that to protect human volunteers and patients, non-animal alternatives do not, as yet, provide enough information to replace all animal studies.

A retrospective assessment of replacement will be due by 09 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated need for use to a similar extent.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

There is no definitive guidance from government regulators on the numbers of animals to be used in the studies described in the project; the applicant and colleagues will use their extensive experience of related programmes, taking account of statistical significance and scientific advice as necessary, to use sufficient animals for studies to provide robust results. Generally group sizes are minimal in nature, using up to 4 animals per group or end-point.

In some studies it is possible to give more than one test item to animals at the same time, and be able to analyse the results, typically the blood levels of the different test items, thereby reducing the total number of animals used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies may be used to investigate the potential of new designs or processes to improve outcomes, before being used in larger numbers of animals. Screening studies, using small numbers of animals, are designed to identify and eliminate materials with undesirable results, and so reduce the numbers of animal which are then used in the studies required by government regulators.

The extensive re-use of some animals enables an enormous reduction in the total number of animals used in the project.

A retrospective assessment of reduction will be due by 09 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Dosing of potential new medicines is by the same way as they would be given to people; most commonly by mouth, but including various injection methods, occasionally dosing by inhalation and by application to the skin. The methods used are generally very well established and commonly used by experienced staff at the establishment. Volumes of drugs to be given are in line with published guidance on minimising discomfort, and/or are known to cause minimal discomfort based on extensive experience at the site.
Blood sampling is a common need. We follow published guidance on methods and suitable volumes which can be taken while minimising harms to animals.
Restraint or confinement of animals is a common need, to allow collection of samples, generally urine and faeces. Methods used are those with which staff have extensive experience, and the duration of time is minimised wherever possible while allowing completion of the process so that tasks do not generally have to be repeated.
Surgery is required for a small percentage of the animals to be used in the project. It is conducted with expert veterinary involvement in the creation of suitable regimes for anaesthesia and post-surgical pain relief.

Why can’t you use animals that are less sentient?

The species used are selected to answer the scientific questions and enable collection of results which will allow continuation of development of the most appropriate new medicine candidates. They are the same species as are used in follow on studies required by government regulators.
Tests are generally of mild severity for animals, and samples are required over a time period which would make continued anaesthesia impractical in almost all cases, would interfere with the outcome in some circumstances.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinement of on-going procedures is commonly discussed and explored within the animal technical, veterinary and scientific groups, and also as and when any concerns are identified; for example additional assessments may be included based on initial outcomes.

The surgery and anaesthesia/pain relief protocols used in the programme undergo regular and routine assessment and refinement to improve outcomes. Habituation of animals to restraint is a routine process, and the schedule can be amended in response to outcomes for individual animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Dose volume and blood volume limits agreed with the animal welfare and ethical review body are based on the 2001 publication of Diehl et al: A good practice guide to the administration of substances and removal of blood, including routes and volumes.
Welfare end-points are developed in general line with publications on the topic, including the NC3Rs document from 2010 on dose level selection for regulatory toxicology studies.
Non-human primate housing is in compliance with the NC3Rs document on this topic from 2017.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Both our clients and our colleagues working in the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which propose refined approaches to conduct of work. **A retrospective assessment of refinement will be due by 09 April 2026**

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

146.Physiological and neurobiological functions of the Trappc9 locus in mice.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Mice	adult, pregnant, neonate, juvenile, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to analyse the phenotype of *Trappc9* knock-out mice as a model for a human neurodevelopmental disorder that is associated with *TRAPPC9* mutations. The symptoms/phenotypes mainly relate to microcephaly, intellectual disability, obesity and behavioural dysfunctions.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This project consists of basic research to improve our understanding of the (neuro)physiological functions of the *Trappc9* (*Trafficking protein particle complex subunit 9*) gene, which is essential for normal human brain development. Mutations of the *TRAPPC9* gene cause a neurodevelopment disorder from childhood onwards, which is characterised by intellectual disability, speech impairment, small brain, delays in various developmental milestones and obesity. Little is known so far about the cellular roles of the Trappc9 protein and how it exerts essential functions in specific tissues like the brain. Trappc9 is involved in intracellular vesicle transport processes and the regulation of lipid storage droplets, but the disease mechanisms of Trappc9 deficiency are unclear. So far, no *Trappc9* knock-out (KO) mouse model has been published, but we obtained such a mouse model through an international consortium to phenotype mouse mutants for all genes. Over the duration of our previous project licence, we have obtained an initial assessment of the *Trappc9* KO phenotype, which confirmed several of the human disease symptoms, e.g. small brain and obesity, thus showing the relevance of the mouse model for understanding the human disorder. This project aims to continue and expand the phenotyping work,
e.g. with regard to behavioural test, imaging (brain MRI) and obesity physiology. The mice will also be crucial for undertaking cellular studies, for example in primary neuron cultures, to investigate the cell-biological functions of Trappc9. Insights gained from this programme of work will shed light on essential mechanisms of brain development and maintenance of neural cell functions, which will also be relevant to human physiology and neurobiology.

What outputs do you think you will see at the end of this project?

We anticipate to obtain several primary research publications from this programme of work. PhD students are currently using the *Trappc9* mouse line in their projects and will achieve first-author publications from their work. Such publications should form a good basis to attract additional grant funding for the conditional knock-out objective, which should lead to follow-on publications later on. It is also likely that future PhD students will join this programme of work and produce additional data. Results will also be presented at scientific conferences organised by academic societies. Furthermore, our mouse phenotyping data will most likely be incorporated into the public database of the international consortium, which originally generated this mouse line for the scientific community.

Who or what will benefit from these outputs, and how?

In the short-term, it is mainly the scientific community working in basic research of neurobiology, cell biology and mouse genetics, who will gain new knowledge about the functions of the *Trappc9* gene in mammals. Our findings in the KO mouse model will also be of interest to medical scientists and geneticists, who are trying to understand rare human genetic disorders and the associated disease mechanisms.

In the long-term, our work might contribute to the knowledge about the molecular and cellular mechanisms of the rare human *TRAPPC9*-related neurodevelopmental disorder, so that therapies might be developed or adapted, which could mitigate at least some of the disease symptoms. Such considerations would be within the remit of the UK government-supported initiatives on research into rare diseases.

How will you look to maximise the outputs of this work?

Some of this programme of work is already undertaken in collaboration with other experts, i.e. the MRI analyses are carried out with the help of our pre-clinical imaging centre. Technical details of this work will, therefore, also feed into the MRI imaging community. We also intend to set up collaborations with colleagues in the behavioural analysis field, with whom I have collaborated previously on highly specialised behaviour tests, e.g. at another UK University and a research institute in Europe.

Apart from presenting our findings as posters or oral presentations at international conferences, we will also publish pre-print manuscripts on open-access servers, which allows public scrutiny and feedback on our results by anybody interested in the work. This gateway will also enable us to communicate unsuccessful approaches or ‘negative results’.

Species and numbers of animals expected to be used

- Mice: Over five years we estimate that we need to use up to 2500 mice for the breeding and maintenance of the genetically modified mouse strains. For experiments, we estimate that we might need altogether up to 600 mice. Most likely, fewer mice will be needed eventually.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Using a genetically modified mouse model to investigate the mechanisms of a human inherited disease that causes intellectual disability and a small brain :

Recent clinical studies have shown that a specific heritable brain disorder is caused by defects in a gene called ‘Trafficking protein particle complex subunit 9 (TRAPPC9)’. Patients, who inherited such gene defects from their parents, show the following major symptoms from childhood onwards: intellectual disability, inability to learn to speak more than a few words, small brain size and delays in developmental milestones like learning to walk. Most also become obesity. Little is known so far about the roles of this gene, and what exactly goes wrong in brain development and function when there is a defect in TRAPPC9.

Mice are a mammalian species that can be readily genetically modified to model human heritable disorders, but so far no mouse model with a defect for Trappc9 has been described in the scientific literature. We established Trappc9-deficient mice to analyse whether they show similar symptoms as human patients and to investigate the mechanisms of the disease. As a mammalian species, mice are still quite similar to humans. We found that, when compared to normal mice, these Trappc9-deficient mice also have a smaller brain when they are young adults, but not at the newborn stage. We are interested in finding out when exactly the differences in brain size start to occur and what goes wrong on a cellular and molecular level in the mouse brain. Therefore, we are interested in analysing weaning-stages, juveniles as well as older adult mice. As an approach to investigate ‘intellectual disability’, we intend to undertake behavioural tests with the adult mice, e.g. memory tests. We also found that the mice show another adult disease symptom, namely obesity, and we are planning to investigate the fat tissue and the brain regulation of body weight, food intake and overall energy balance. We know, that other laboratory animal species with defects in Trappc9, i.e. the fruit fly and the roundworm, do not show the human disease symptoms and are therefore not a good animal model to investigate the disease.

Typically, what will be done to an animal used in your project?

Typical experiments that we are planning to do include the following:

Cohorts of Trappc9-deficient mice and their normal littermates will be kept for up to one year and their brains imaged via MRI scans at various ages. For this, the mice will be transiently anaesthetised, from which they recover very well. We can measure the brain volumes and structural differences in brain sub-regions with these MRI data. Additionally, the cohorts of mice will be used for testing in several behavioural tasks, e.g. exploration of a new environment, locomotor activity and coordination, memory tasks. We can then correlate our brain MRI data from individual mice with their behavioural performance.

Other cohorts of mice might be used to explore whether brain stem cells are affected by defects in Trappc9. This could be an explanation for smaller brains. A typical experiment in this context would be to inject a dye into the abdominal cavity, which then gets distributed throughout the body and labels stem cells in all tissues. The brain tissue will then be collected and sections analysed under the microscope to quantify the number of brain stem cells.

In further cohorts of mice, we intend to analyse the obesity symptoms. This includes measuring food intake, blood glucose, insulin and other hormones. Depending on initial findings, we might also inject (via various routes) substances that have an influence on whole-body energy balance regulation, including regulation of food intake. We intend to find out whether the obesity is due to a role of Trappc9 in specific brain regions that control energy balance, or whether it is a defect directly in fat tissue.

In other experiments, we will need mice to obtain tissues for molecular biology analyses, and to grow cells in culture in the lab, for example neurons or fat cells. The cell culture experiments will give us important information on a microscopic level, i.e. which structures and molecular mechanisms inside the specific cell types are not working normally when Trappc9 is missing.

What are the expected impacts and/or adverse effects for the animals during your project?

From the analysis of brain stem cells, we do not expect any major adverse effects on the mice. The substance that labels the stem cells does not normally cause adverse effects over the duration of the experiments. In the long term, there is a possibility that it might cause cancer, but we are not keeping the mice for long enough after the injection for cancer to develop.

In the MRI experiments, mice will be kept under gas anaesthesia for relatively long periods, e.g. up to 6 hours. Their body temperature, breathing and pulse will be measured constantly and maintained continuously. Also, a protective cream will be put on their eyes, so that they don't dry out. Mice usually recover quickly from the anaesthesia. The behavioural test should not cause more than occasional anxiety. We intend to only use experiments in dry environments (no swim tests) that do not cause major adverse experiences.

In the context of analysis of obesity, some of the experiments might cause adverse effects in the longer term, e.g. feeding a high-fat diet will make any obesity symptoms even more extreme. But we are not planning to keep such mice over many months; they will be terminated as soon as the experimental objectives are achieved. Testing for insulin sensitivity (via injection of insulin and sampling of blood glucose levels at regular intervals from a superficial vein for approximately two hours) can in rare cases cause hypoglycemia. However, this would then only occur in lean mice, not in obese models, and in such a case the mice will immediately be given a glucose injection to counterbalance the insulin effect. We also intend to undertake glucose tolerance tests, i.e. injection of glucose and measuring the rise and fall of blood glucose levels over a two hour period. This should not cause any adverse effects. If we find that brain dysfunctions are the major cause for the obesity of Trappc9-deficient mice, we might test the effects of substances that influence the regulation whole-body energy balance through injection via appropriate routes. In this case we will measure the short-term effects of the substances within hours or a few days, e.g. effects on food intake, or on molecular biological markers in brain tissue, for which the mice would be humanely killed at defined time points and tissues harvested for analysis.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Overall, the majority of mice that are used in experiments will only experience a mild severity level of intervention. A smaller proportion of animals will experience moderate severity interventions.

In the analysis of brain stem cells, ~70% of mice will only experience mild peripheral injections of a labelling substances. However, under some circumstances we might want to investigate the effects of growth factors that keep stem cells alive or stimulate them in other ways. Such factors might need to be injected into the brain or its ventricles. For this, we would need to undertake surgical procedures to place a cannula into the brain. There is no pain sensation in brain tissue itself, however there are risks of complications from the surgery, e.g. infection, which would class this procedure as moderate.

All mice that undergo MRI brain scans will be classed as experiencing moderate severity. This is due to the length of the imaging procedure (up to 6 hours), which is carried out under gas anaesthesia. Mice usually do recover without problems from such longer periods of anaesthesia, but body temperature, pulse rate and breathing need to be monitored and maintained during the scans. All behavioural test are within the mild severity category. Some mice (~30%) might be used for behavioural tests only, without MRI data being acquired.

We estimate that approximately 50-60% of mice that will be used for the analyses of energy balance and obesity might experience moderate severity. This would include development of more extreme forms of obesity due to high-fat diet feeding, moderate transient hypoglycaemia during an insulin sensitivity test and injection of energy homeostasis-regulating substances (potentially including injections into brain / brain ventricles).

What will happen to animals at the end of this project?

- Kept alive
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The human inherited disorder caused by defects in the TRAPPC9 gene leads to symptoms of intellectual disability, a smaller brain, inability to learn to speak, obesity and other symptoms. These are abnormalities in complex tissues and interacting organ systems of the body, which cannot be fully understood without an animal model. Also, some of the human disease symptoms appear to be due to processes occurring during the late maturation stages of brain development. Complex processes like the development of an organ can currently not be modelled outside of an animal. The best mammalian system to model such human gene defects is the mouse, which can be genetically engineered to recapitulate the human genetic abnormality. Its brain is still similar enough to the human brain in many aspects, to enable conclusive insights into the mechanisms of disease. A mouse model will allow us to analyse the development of the brain over time, do behavioural test related to intellectual disability (such as memory tests), analyse hormone interactions and whole-body energy balance regulation.

Which non-animal alternatives did you consider for use in this project?

Some functions of Trappc9 within cells can be analysed to some extent in vitro in cultured cells, and we are undertaking such experiments in parallel to the animal work. For example, we are using cell lines in culture to investigate specific structures inside the cells, termed lipid vesicles.

Why were they not suitable?

We found that standard, permanently used cell lines have their limitations, since they are not the same as the cells that are mainly affected in the human TRAPPC9 genetic disorder, namely nerve cells in the

brain (neurons). Neurons are highly specialised cell types, which (unlike standard cell lines) do not multiply further. They have sensitivities that are not found in standard cell lines, for example regarding lipid vesicles, which appear to be regulated by Trappc9. A defect in such lipid vesicles seems to have minor impacts for standard cell lines, but can be toxic to neurons. For this reason, we found that neurons directly isolated from mouse brain tissue and maintained in culture dishes in the lab are a truer model for the disease symptoms.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

I have estimated these numbers of animals based on my previous experiences from maintaining and investigating genetically modified mouse models of human disease. The estimates of experimental animals required are also based on experience from past and/or currently ongoing experiments that are similar to what is proposed in this project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use the NC3Rs Experimental Design Assistant (EDA) to design animal experiments, maximise the data readout from a minimal number of animals and obtain estimates of how many mice might be needed for a specific experiment. I am also familiar with Power calculation software and regularly use the freely available GPower software
for the estimation of required sample sizes in experiments.

For experiments where we cannot use formal software in advance to estimate sample size, for example in novel experiments where we do not have an estimate of the expected standard deviation, we will utilise a block design or the similar ‘variable-criteria sequential stopping rule’ (SSR), whereby a subgroup (small number of wild-type and knock-out littermates) is used at a time initially. Data from several such subgroups will be collected over time and added to the total experimental N. Data will be analysed intermittently after addition of subgroups for statistical significance and Power.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To minimise the numbers of animals produced in the maintenance breeding of the genetically modified mouse lines, we will set up a limited number of breeding pairs intermittently, i.e. three times a year. Constant breeding throughout the year will be avoided, to not produce unnecessarily large numbers of excess offspring.

Furthermore, wherever possible, we will offer tissues that we do not need for our own research to other interested scientists in order to share resources and maximise the usage of the animals for multiple aims.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will be using genetically modified mouse strains that correspond to a human inherited disorder, which is due to a specific gene disruption and causes neurodevelopmental symptoms. It is the only animal model, which reproduces the complex human disease characteristics.

As described in detail in the above section ‘Anticipated Harms’ we will be using the most appropriate methods, experimental approaches and techniques to minimise pain, suffering, distress and/or lasting harm. Data read-outs from cohorts of experimental animals will be maximised wherever possible, for example by using the same cohorts for brain MRI scans and behavioural tests over a period of time.

Why can’t you use animals that are less sentient?

We considered the roundworm C. elegans as an alternative model, but found out that lack of Trappc9 in worms results in lethality. Another laboratory animal, the fruit fly Drosophila, does not show brain abnormalities when Trappc9 is inactivated. The symptoms are related to different tissues in this animal.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For many experiments in this project, we do already have experience from previous work and have established ‘best practice’ protocols, e.g. for the MRI brain scans and the associated regular monitoring of recovery from extended periods under anaesthesia. For other experiments, standard protocols are published and widely used in the scientific community, e.g. for glucose tolerance and insulin sensitivity tests, or for the stem cell labelling experiment. Here, the minimal doses and concentrations of substances that are required to achieve the aims will be used. When surgical procedures are used, best sterile practice will be implemented in our dedicated surgery rooms, and analgesia or other pain/distress-relieving measures implemented with the advice of our NVS.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Protocols, training resources and guidelines on best practices in animal experiments are available through the NC3Rs website, including handling and restraint, euthanasia, humane endpoints, welfare

assessment, micro-sampling, anaesthesia, analgesia. Personal licence holders, who are involved in this project, will be asked to familiarise themselves with this information.

We will follow the ARRIVE guidelines as recommended by the NC3Rs.

The Grimace scale of facial expressions, which is also published on the NC3Rs website, will be used to inform on animal pain condition in mice
.

The NC3R online Experimental Design Assistant will be used when new experiments are planned.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I am receiving the regular NC3R newsletters via email and I am checking the NC3R websites for novel developments in the field as well. The representative of the NC3Rs for our region visits the University regularly and organises events, presentations and webinars that are well attended. Furthermore, the head of our animal facility and our local Animal Welfare and Ethical Review Body forward information on novel developments in the field to all licence holders.

Home Office

NON-TECHNICAL SUMMARY

147. Plasticity after injury to the nervous system

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Pain, Nerve Injury, Spinal Cord Injury, Plasticity, Neurotoxin

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The nervous system undergoes continuous change in terms of the structural connections between nerve cells,

the strength of these connections and how readily signals are generated. These changes are termed plasticity and they adjust and regulate the function of the nervous system according to changing requirements throughout life. When the nervous system is damaged, for example if a peripheral nerve is cut or a high velocity impact results in a spinal cord injury, this ability of the nervous system to change can help it to adjust and compensate for the damage, and helps to provide a degree of functional recovery. However, there are limitations to these plastic changes which in turn limit the degree of functional return. We aim to investigate these so that we can better understand how return of function might be optimised. We will also investigate the effect of drugs that increase the plasticity to see if these can promote improved recovery.
Plastic changes after injury to the nervous system can also have negative consequences. After both peripheral nerve and spinal cord injury, there can often be the development of a particularly unpleasant form of continuous pain. Because it is caused by damage of the nervous system, it is called neuropathic pain. This sort of pain is very debilitating and particularly difficult to treat as drugs are often ineffective. We do not fully understand why this maladaptive, pain producing plasticity occurs but we now have tools and approaches that can help us understand the circuits of neurons responsible, and how they change. The signalling of pain by the nervous system also involves several areas in the brain and one such area has only recently been identified. It will also be important to define the role of changes in these brain areas to the development of chronic pain. There are now also agents that have the potential to interfere with signalling in spinal cord and brain circuits and these may prove useful in preventing or blocking the pain signal. One group of such substances are nerve toxins. This project aims to provide a better understanding of the changes in spinal cord and brain circuits that lead to pain conditions and test whether these substances can successfully block pain. We will also investigate whether these neurotoxins can prevent the over-activity in the nervous system that leads to dangerously high blood pressure (autonomic dysreflexia) and to painful muscle stiffness (spasticity) in spinal cord injured patients. The toxins will be used at levels well below those that cause muscle weakness or other adverse effects.
A retrospective assessment of these aims will be due by 17 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The aim of the project is to improve understanding of the plastic changes in the nervous system that occur after it is injured. The potential benefits of the work are that this understanding could provide the basis for developing better treatments for restoring sensation and movement; and improved drugs for controlling the debilitating conditions of chronic pain, dangerous increases in blood pressure and painful muscle stiffness.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The project will use rodents, both rats and mice. It is estimated that the experimental programme will require approximately 1,550 rats and 1,650 mice over the course of 5 years. In addition, 5,350 mice will be used for breeding purposes only.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

More than half of the mice will be used to for breeding and maintenance of genetically modified lines, and since the vast majority of these animals will have no behavioural abnormality, this is classified as "mild". A small proportion (estimated at 5%) of the animals (rats and mice) will undergo procedures that are carried out under general anaesthesia, from which they will not recover, and these are therefore classified as "non-recovery". A further group of animals (estimated at 45%, predominantly mice) will undergo procedures such as injection of harmless tracer substances into the brain or spinal cord, or spinal injections of agents that will activate or inactivate different nerve cell populations. These procedures are performed under general anaesthetic. These animals will receive post-operative painkillers and should experience no more than transient discomfort resulting from the operation. About 50 % of animals (rats and mice) will either have a nerve injury (a moderate procedure) or spinal cord injury operation (a moderate or severe procedure), both of which may lead to a mild form of pain with increased sensitivity to touch or warm stimuli. The animals' ability to eat and drink will not be restricted. After nerve injury and spinal cord injuries that are made with a device that applies an impact (contusion) there may be some lameness but animal’s movement is not restricted. In a small proportion of the animals (estimated at 1%, rats only) the spinal cord will be completely cut. This is a severe procedure.
These animals have a temporary inability to empty their bladder and need to be regularly assisted. They also permanently loose the use of their hind limbs. These animals feel no discomfort during the application of stimuli to test for autonomic dysreflexia or spasticity, as these are applied below the injury where there is no sensation. At the end of the procedure the great majority of animals (estimated to be >95%) will be killed whilst they are under the influence of a general anaesthetic. A much smaller proportion will be killed by another humane method.
A retrospective assessment of these predicted harms will be due by 17 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The objectives can only be achieved by experiments using live animals because the complexity of the biological systems to be examined, and the need for events to develop consequent to manipulations such as injury cannot be reproduced *in vitro.* Neural circuits and changes in connectivity due to plasticity all require investigation in intact animals. Conditions such as pain, spasticity and altered cardiovascular function cannot be modelled in other ways. Where possible, however, our work will be informed by in vitro approaches. This applies in particular to development of agents for neural repair. In addition, we incorporated into our Project Plan, the use of ex vivo preparations. **A retrospective assessment of replacement will be due by 17 January 2026**
The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

The numbers of animals to be used in each part of the study will be the minimum required to provide reliable results (avoiding as far as possible inter-animal and technical variability). Many of the outcome measures that we obtain are based on observation and are largely qualitative or can only be treated in a semi-quantitative way.

For these types of results, we will collect results from a minimum of 3 biological replicates. Where appropriate results will be analysed statistically for significance. In addition, where data is available we will use power calculations which is a method for predicting the numbers of animals that are required to provide statistically significant answers. Experimental design is continually reviewed and our studies carefully executed in order ensure use of minimal animal numbers consistent with the experimental aims and to maximise information obtained from each animal.
A retrospective assessment of reduction will be due by 17 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Rodents are the most appropriate species as they are the least sentient animals that could be used. Non-mammalian species show a capacity for nervous system regrowth which does not happen in mammals and also show much greater plasticity. The larger size of rats makes some procedures such as fMRI more feasible. Nevertheless, mice may offer an advantage where genetically altered lines are available because this allows for the targeted manipulation of the responsiveness of particular groups of neurons.
We have experience of all of the models to be used and a good understanding of both their scientific merits and the animal husbandry required to minimise adverse effects. Together with the Veterinary and Biological Services staff, we regularly review the general procedures for animal care including analgesia, fluid replacement, and post-operative maintenance of body temperature. In addition, for more severe injuries such as spinal cord contusions and transections, we have introduced a monitoring chart, which incorporates built in end points. I collaborate widely with others to ensure that we follow the most modern and refined approaches to these severe models of disease and I contribute to published material in this area. All animals will be group housed wherever possible. **A retrospective assessment of refinement will be due by 17 January 2026**
The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

148. Pluripotent stem cell derived hepatocytes for treatment of liver failure

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

liver diseases, acute liver failure, mouse model, stem cells, therapy

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Stem cells are special cells in the body from which all other cells develop. The aim of this project is to find out whether stem cell products could be used as a potential treatment for liver failure and inherited liver diseases, by transplanting them into rodents with these liver diseases and assessing whether the cell therapy aids recovery.

A retrospective assessment of these aims will be due by 07 March 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Liver failure and liver diseases are life threatening human diseases for which currently the only long-term treatment is a liver transplant. Due to a shortage of liver donors, for many patients this life saving treatment is simply not available. Furthermore, there are a number of long-term risks for recipients, including organ rejection and unpleasant side effects from the drugs that have to be given on a permanent basis to prevent rejection. Stem cells afford us the exciting possibility of being able to cure patients with liver failure where otherwise the only proven way of treating them is whole organ transplant. Before such cells can be used in patients it is an essential requirement that their effectiveness is tested in animals which model the human disease, and are proved safe.

What outputs do you think you will see at the end of this project?

This research could demonstrate the effectiveness of a potential new therapy for patients with liver failure and inherited liver diseases, and so benefit thousands of patients worldwide. Liver diseases comprise a significant and increasing clinical burden. The current standard treatment for liver failure is whole organ transplant, but the number of patients requiring transplant far exceeds the number of available donor organs making alternative treatment methods an urgent priority/clinical need. The short term benefits of this project would be confirmation that stem cells are potentially an effective, safe treatment for liver failure in rodent models.

Who or what will benefit from these outputs, and how?

At the end of this project we expect that we will have proved our idea that these cells are an effective therapy for liver diseases. The new therapy will then be further developed for approval by the Medicines and Healthcare products Regulatory Agency (MHRA)) for clinical trials in humans (medium term benefit). Should Phase 1 trials be successful then these cells could be given to patients in the long term and extend lives of patients with liver failure who are not selected for or cannot have transplantation surgery. In the longer-term, stem cells could replace organ transplants as a more readily available method of rescuing the failing liver without the need for complicated surgery.

How will you look to maximise the outputs of this work?

The outputs from this work will be shared through publication and by presentation at conferences, and through our partner biotechnology company, who will develop this further as a proposal to the MHRA with a view to using them on patients in a hospital setting.

Species and numbers of animals expected to be used

- Mice: 7500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice have been chosen as the experimental animals of choice because the basic features of their livers are remarkably similar to other mammalian species and of are similar complexity to the human.
Immunocompetent mice (such as C57BL/6) will be used in the study of optimization and identification of therapeutic efficacy of PSC hepatocytes of this project, as they do not reject the encapsulated human cells.
Genetically altered mice with liver injury will also be used. Human liver cells are inserted into their livers, providing a means of testing our stem cells against failing human liver tissue.

Typically, what will be done to an animal used in your project?

Some animals will be bred to produce animals with immune systems which do not work properly or with liver damage.
Most animals under this licence will undergo liver injury to bring about liver failure. Animals may have surgery to cut out part of their liver tissue or be dosed with chemicals which damage the liver. Some animals with inherited liver disease problems may also be used.
Stem cells will be delivered either into various sites on the belly or placed under the skin to try to rescue the failing liver. Blood samples and scanning will take place to monitor how the liver is working and movement of the cells we have introduced.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals are expected to get sick and some with liver failure might die, they will be monitored very frequently so that they can be humanely killed where possible, to prevent further suffering if their liver failure is not responding to the stem cell implants.
Advice from the Veterinary surgeon will ensure that the welfare of the animals is maintained at the highest possible level for their condition. Any animals that show unexpected side effects of the liver injury or of treatments will be killed to avoid unnecessary suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The breeding strains are expected to experience mild severity (70% of animals) or moderate (30% of animals) severity on this protocol.
Animals undergoing protocols to generate liver failure are likely to experience severe signs (up to 80% of animals), and some may need to be humanely killed to prevent further suffering. The disease develops very quickly and a proportion of animals may die before they can be given any treatment.
Animals undergoing studies on inherited liver diseases are expected to experience moderate severity (100% of animals on this protocol)

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 07 March 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Unfortunately there are no alternative techniques that could replace the use of animals in this project. In order to demonstrate that stem cells would be effective if used as therapy for liver diseases, they have to be tested in animals with the diseases. It is also a requirement for the techniques to be tested in animals and deemed safe before they can go on to be developed for use in real patients.

Which non-animal alternatives did you consider for use in this project?

Our approach is to use experiments in the laboratory as much as possible to replace animal experiments. Initially, laboratory testing will provide information on what cells actually do, thereby decreasing the numbers of animal experiments by screening for the most useful cell lines.

Why were they not suitable?

Laboratory methods are suitable for initial screening of cell lines for treatment potential.
These methods cannot however adequately model the all of the things cells would be exposed to in the entire animal, making animal based testing a necessity. Importantly it is a requirement of the Medicines and Healthcare products Regulatory Agency (MHRA) that products from stem cells such as these be tested in animals prior to use in human patients.

A retrospective assessment of replacement will be due by 07 March 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We will endeavour to use laboratory based methods wherever possible thus limiting the numbers of animals undergoing experimental procedures.
The sizes of experimental groups and the number of repeated experiments will be kept to a minimum while ensuring that reproducible results are obtained with clear biological significance. We use statistical analyses to determine the minimum numbers of animals needed for our studies that will produce robust results. We have estimated our initial group sizes from laboratory studies, which suggest that group sizes of 3 might be sufficient to detect experimental effects. A greater degree of variation is expected in animal studies compared to that observed in the lab and we anticipate that we will need group sizes of 6-12 for our animal experiments. This will be revised as the animal work under this project progresses.
We aim to further refine our techniques through high success rate of surgical procedures and good practices of performing preliminary experiments to establish the minimum number of animals. Furthermore, we will continue reducing the use of animals as much as possible in this licence, in collaboration with the named veterinary surgeon.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Advice in experimental design has been taken locally from statistician and scientific colleagues.
Online tool (the NC3R's Experimental Design Assistant) has been referenced.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Cells to be used for implantation will be screened in the lab before they are used with animals, to ensure that they are likely to produce the effects which we are looking for.
Studies using small numbers of animals will be undertaken in initial work to determine the best methods for cells to be implanted before full scale experiments in animals with liver failure.
Where more animals are bred than we need, surplus animals will be made available to others for tissue sharing where possible.

A retrospective assessment of reduction will be due by 07 March 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse model has been chosen as the experimental animal of choice as the basic features of their livers are remarkably similar to other mammalian species and have a similar level of complexity to the human liver. Mouse models of liver failure and inherited liver disease are successfully used elsewhere in this area of research, can be reproduced and represent relevant models of the range of human diseases we are studying
Immunodeficient (less able to recover from infection) mice are necessary for some studies in order to avoid the human cells/tissues we are introducing from being attacked by the body. These animals will be subsequently bred in house.
The mouse paracetamol and partial liver removal model is the standard model of acute liver failure. Mice are also reliable models for our area of research.
Genetically altered mice provide reliable and relevant models of inherited liver disease which can be brought about as required, animals remaining normal until the disease is 'switched on'. Genetically altered mice which have, or can be made to have liver failure, do not require an initial surgery or toxic dose of chemical before stem cells are delivered. The humanised livers of these mice additional provide a method of testing these stem cells against damaged human liver cells.
For these reasons, mice are the species of choice for our studies. These animals are needed to investigate how human liver cells that have been made from stem cells work and how they can ease liver failure symptoms. The mouse models are important in providing new information and as models in which to study new ways of transplanting cells.
Suffering
All the protocols proposed in this application use well-established and tried techniques that have been refined to involve a minimum of suffering. Sterile surgery will be conducted by researchers with considerable experience in animal techniques; training and competency records will be kept. Attention will be paid to keeping rodents warm and hydrated during surgery to improve outcomes. Anaesthesia and pain relief will be administered to minimise discomfort and the animals will be assessed at least daily for any signs of distress. Experiments with a small number of animals will take place first i to refine methods, identify effective doses and avoid excessive deaths. In all the proposed animal experiments, if animals display signs of distress, advice will be sought from the Named Veterinary Surgeon (NVS) and, if distress cannot be eased, the animals will be humanely killed.
Liver failure is associated with a significant death rate and all attempts will be made to avoid this. Animals will be observed multiple times per day after we have brought about liver failure, and small samples of blood will be taken to check liver function using markers in the blood. Hand held blood analysers, as used in human patient care,will be trialled in an attempt to identify animals whose condition is deteriorating quickly so they can be humanely killed immediately rather than waiting for samples to be processed remotely in the lab.

Why can't you use animals that are less sentient?

The livers of non-mammalian species do not have the same make up as human livers are not good models of human liver disease as they relate to human patients. In addition, animals which have been bred not to reject the transplanted cells are needed for some studies, and these are generally only available in rodents.
The ability of the liver to grow new cells is less in younger animals, thus younger animals are unlikely to recover from liver injury and are not suitable as models for the human adult patient. The mouse is considered least sentient mammalian species and will be used for the majority of studies.
It is not possible for us to carry out our studies on anaesthetised animals which are killed without waking, as we need to find out how the animal recovers from liver failure.
The ability to genetically alter mice also makes them our species of choice.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We aim to develop blood tests to indicate increasing likelihood of severe outcomes such that animals can be identified at an early stage and suffering minimised.
We will use frequent observations of animals throughout the critical phases of each experiment, measuring body weight and condition and observation of eating and drinking.

Our staff have considerable experience in normal animal behaviour and we will take advice from our named people on animal welfare.
We will start with small scale experiments to determine the most efficient and best way to carry out our studies, progressing to full scale experiments once we have improved our understanding of the unknowns.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will make use of published information on methods and use resources such as PREPARE and the NC3Rs to help us keep informed about best practice.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will communicate with our named people throughout the project and seek advice on refinement of procedures. We well attend conferences and 3Rs events and implement any improvements to the protocols that are identified.
We will also report back to our local AWERB and our Inspector after our initial experiments to determine doses.

A retrospective assessment of refinement will be due by 07 March 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

149. Preclinical targeted therapeutic approaches in colorectal cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:

Key words

No answer provided

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Bowel cancer, mouse models, therapy.
In the UK, colorectal cancer (CRC) is the fourth most common cancer representing approximately 12% of all new cancer cases (CRUK, 2015). Although the introduction of screening programs has led to higher rates of detection at early stages of disease, more than 50% of people are diagnosed at a late stage. Moreover, the treatment options for these patients has not significantly improved over the last 20 years. New targeted

therapies have been developed but unfortunately these have not translated into better survival, primarily because trials failed to stratify patients accordingly. Therefore we plan to use pre-clinical models of CRC to inform the design of clinical trials for targeted therapies in human bowel cancers.

Our objectives are:

1) Generate models of colorectal cancer incorporating genetic features of human disease, and representing clinically important patient populations

2) Use these models to identify, develop and test new therapeutic targets.

3) Use this information for the design of stratified clinical trials to directly benefit human patients.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Through the research outline in this project, we will establish the use of patient-relevant models of colorectal cancer for the preclinical testing of novel therapeutic approaches. The project will allow for analysis of the impact of novel therapies in the context of tumours driven by oncogenic mutations which represent A) the most common mutations found in colorectal cancer and B) the oncogenic mutations which represent the most pressing clinical need in colorectal cancer at present.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We expect to use around 102,500 mice over a 5 year period. These mice may carry the genetic changes known to cause bowel cancer.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Mice with genetic changes which are known to be critical to formation of colorectal cancer in human patients will be bred under this licence. Of these, approximately 50% of the animals will not show any adverse effects and will only be subject to ear notching for identification purposes and for identification of genetic changes. Approximately 20% of animals will be pre-disposed to colorectal cancer - these will be continually monitored for clinical signs indicate tumour growth. These signs include paling feet because of anaemia, weight loss, swelling of the abdomen and development of palpable tumours. These symptoms will be monitored for by trained staff and if the tumours interfere with normal behaviour, reach the limits of the size allowed in the guidelines or have any consequences that are out with the guidelines, mice will be humanely culled and tissues harvested for

analysis. Tumour growth in mice may also be monitored using advanced imaging techniques which are relevant to human patient experience, such as MRI, ultrasound or CT scanning, which involves anaesthetising the mice for short periods of time during which images can be collected. Animals which have intestinal tumours may be administered with treatments design to reduce tumour growth, which have the potential to benefit human patients with colorectal cancer. These treatments can include drugs, which are administered by injection, pellets under the skin or orally, or radiotherapy, using a specially designed small animal radiotherapy machine. In some cases, the diet of the mice may be altered to promote tumour growth, or to enhance the effect of treatment, All treatments are performed by highly experienced staff, and all mice on treatment will be monitored closely for any signs of ill-health.
Tumour cells will be grown in the laboratory and may be transplanted into mice, to model colorectal cancer. These may be injected under the skin, or following general anaesthetic, directly into the colon using an endoscope guided injection to represent early tumour growth or into the spleen or liver to represent metastasis. All study animals will be killed by a humane method and tissue specimens taken for analysis after death.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Colorectal cancer is a complex disease which involves interaction of a number of different cell types such as tumour cells, immune cells, and blood vessels. Also the environment the tumour is within plays a role in its, growth, development, ability to spread and how it will respond to drugs. There are currently no non-animal models that can reproduce this environment correctly and therefore they are not useful for studying colorectal cancer progression and the effectiveness of new therapies. Moreover there are now therapies that can reactivate the patient's own immune system, which can only be tested in animal models with a functioning immune system. To inform our experimental design and reduce the number of animals required we will perform preliminary drug studies in primary intestinal cell lines in the lab.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will integrate pilot experiments with a small number of animals into cohort studies to minimise the likelihood of unexpected adverse effects related to genetic modification and/or therapy. The numbers required are calculated from our extensive experience of the models, and also using statistical tests to ensure the minimum number of animals are used to reach the statistical significance required. We will share animals between experimental groups e.g. when controls from one colony can serve as controls for another experiment.
We will regularly review our breeding colonies to ensure we are using the lowest number of animals to produce animals required for our studies. We will also perform transplant studies in wildtype mice, where relevant, thereby reducing our requirement for genetically altered animals.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

It is widely recognised that genetic mouse models of colorectal cancer are most closely representative of human tumours. The tumours form in the correct tissue, and follow a similar progression pathway as observed in human tumours. We will use the most up to date technologies that allow us to produce specific models in which the cancer develops in the correct tissue with as few as possible side effects. This is done using mice that produce an enzyme to reduce/activate expression of the genes of interest only in specific tissues.
We also have imaging techniques so we can monitor the growth of tumours in real time. Therefore the response of the tumour to therapies can be monitored resulting in a reduction in the number of animals needed per study. Also tumours can be detected earlier.

Home Office

NON-TECHNICAL SUMMARY

150. Preclinical therapeutic models of ovarian cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

ovarian cancer, therapy, evolution, adaptation, resistance

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Ovarian cancer is a group of different diseases with marked molecular variability both within an individual and between women with the same cancer. This causes variable treatment responses and difficulties in personalizing therapy for the individual patient. We aim to use patient-derived xenografts to discover new more effective medicines and to study how ovarian cancer cells adapt and evolve with time and treatment.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

We will primarily focus on high grade serous ovarian carcinoma (HGSC) which is the most common form of ovarian cancer and is amongst the deadliest of all women's cancer. The main reason behind this poor outcome is diagnosis of the majority of patients at an advanced stage and the complex molecular changes in each cancer. Although most HGSC respond to initial platinum-based chemotherapy, development of resistance to treatment and recurrence is frequent. Unfortunately, there are few effective treatment options for patients with resistant or recurrent disease, showing the urgent need to improve therapeutic approaches available for treatment of HGSC patients. No significant outcome improvement has been achieved over the past 20 years and only ~30% of patients with advanced ovarian cancer are alive at 5 years. Thus there is a need to a) understand the underlying mechanisms of resistance and b) to test new therapies for treatment.

What outputs do you think you will see at the end of this project?

The outputs will be data demonstrating (i) the effectiveness of novel anticancer therapies to be used in window of opportunity clinical trials, (ii) identification of which particular tumours (with specific genetic features) are most likely to respond, (iii) identification of new improved modelling of HGSC and (iv) identification of the best biomarkers that can subsequently be used in clinical trials to measure response. It will also generate new information on the underlying biology of ovarian cancer. These data will lead to scientific publications and may generate Intellectual Property. These data will enable decisions on whether to progress new treatment approaches into clinical trials in patients.

Who or what will benefit from these outputs, and how?

Short term: scientific publications are likely to arise from these studies, building a package of data to justify translation of the best treatments into the clinic. There will also be benefit from identifying those treatments that are not effective or have a poor therapeutic index in vivo, thus avoiding future patients' exposure to non-beneficial treatment. Longer term: these animal studies may contribute to patient benefit by identifying more effective cancer treatments.

How will you look to maximise the outputs of this work?

The proposed studies require collaboration with pharmaceutical/biotech companies who are developing the drugs we wish to test, and we have long-standing and productive collaborations that will continue to give us access to the best therapeutic agents that are commercially viable. Most of the companies will not have ovarian cancer as their primary tumour of interest for their drug(s) and so it is mutually beneficial for us to test their drugs in our patient-derived tumour mice models. We will have access to their scientific expertise and unpublished data regarding the agent and the best ways to measure its effects.

We will continue our existing within-centre and multi-centre collaborations and will disseminate new knowledge via publications, including unsuccessful approaches.

Species and numbers of animals expected to be used

- Mice: 5,735

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Immunocompromised mouse strains are necessary to avoid the rejection of human cells. We will use young adult mice for tumour implantation and drug response treatments as we predict tumours will take a long time to relapse after successful treatment with certain drugs.

Typically, what will be done to an animal used in your project?

Due to the nature of the project the mice will undergo surgical procedures to implant ovarian cancer tissues or cells either subcutaneously or in the ovarian bursa. Also mice will be injected with tumour cells intraperitoneally to promote the development of ascites fluid. Tumour cells implanted in mice could have been genetically modified in vitro prior to implantation. Tumour growth will be occasionally monitored using imaging techniques. Mice will receive treatments to study tumour adaptation and to find vulnerabilities that could be exploited clinically. We will use standard of care drugs used in the clinic such us platinum-based agents as well as targeted-therapy associated with our recently developed copy number signatures. We will treat mice with single and/or combined drugs in such a way that we will minimize the number of administrations (e.g. when possible we will combine two drugs in one injection). Treatment duration will depend on the drug used for example when using olaparib we will administer the drug orally for seven continuous days three times leaving a ‘vacation’ week in between. Carboplatin will usually be administered intravenously for two days with a ‘vacation’ week in between. During treatments the mice will be subjected to biopsy and blood collections to a maximum of 4 times each and these materials will be used for downstream experiments and data collection and analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

We will follow the guidelines for welfare and use of animals in cancer research to minimise the adverse effects if any and use appropriate humane endpoints when needed. Animals will be kept in our designated facilities where they will be maintained and monitored daily for any health concerns. Animals will be anesthetised when tumours are implanted underneath the skin or in the ovarian bursa and full recovery after surgery is expected for most animals. Sometimes animals have post-operative complications such us non-healing ulceration due to the initial surgery and in those cases animals will be humanely killed. Animals experiencing a limitation in movement or normal behaviour due to tumour growth will be killed. Some of the mice could also lose body weight due to the tumours and/or the treatments they receive and this will be monitored carefully by comparing weight to matched healthy animals. Some animals will be treated with standard of care drugs and targeted-therapies and the main routes of administration for these drugs will be oral and intravenous. Oral administration will cause minor discomfort on the animal gut whereas intravenous administration will cause discomfort on the tail but both procedures are quickly done so the actual discomfort would last a few seconds.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

In this project licence we will only use immunocompromised mice and the overall expected severity is 45% of mice to experience moderate levels and 55% to experience mild levels. This proportions are mainly based on the surgery procedure necessary for subcutaneous and ovarian bursa tumour implantations and consequently tumour growth and potential metastasis arising from some of the samples.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are planning preliminary experiments using cell lines and organoids, and we will only use drugs or genetically modified cells that have been demonstrated to work in vitro before progressing to experiments in animals. These in vitro experiments will give a good indication of the potential for working in vivo but there is no guarantee that would be indeed be the case. In vitro systems cannot reproduce the influence of the circulatory system and the complex tumour microenvironment, and understanding the contribution of these systems from the in vivo experiments will become crucial to determining the likely success in the clinic. In addition, the fate of populations of cells may differ when assessed in vivo as compared to in vitro.

Which non-animal alternatives did you consider for use in this project?

We are using our newly established organoid models and also cell lines for many of the preliminary drug tests and also for any lineage tracing or CRISPR genetic engineering techniques to be used.

Why were they not suitable?

Although we have developed a way of growing patient-derived organoids some of the samples we are interested in come directly from biopsy tissue and are therefore very small to be processed as organoids. Also some of the tissues that do not initially grow as organoids will do so after being grown in mice. Ultimately the fate of populations of cells might differ when assessed in vivo as compared to in vitro.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We will reduce the number of animals used by doing preliminary in vitro drug testing whenever possible (e.g. if the cells are able to grow) and also lineage tracing experiments will be first tested and trialled in cell lines.

Another way of reducing the number of animals used on the experiments is by doing biopsies during treatment as this prevents the need to sacrifice the animal at every single time-point to get tumour samples. Also we have developed new methods of tracking drug response by analysing ctDNA changes in blood spot samples.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We sought advice from professional statisticians as they provide support with the randomisation and blinding of the experiment, as well as sample size calculation.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies using cell lines and organoids that will help us to design the best preclinical study for a given drug/s or genetic modification. We will use the NC3R's Experimental Design Assistant in those instances where our standard support is not available.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use immunocompromised mice as we will need cells/tissues to grow for the treatment experiments. The methods applied in this licence for growing tumours and monitoring them during time after treatment have been well-known and used in many labs around the world. Also for the last five years, we have optimized procedures to minimize potential pain, suffering or distress as well as enhance animal welfare.

Why can't you use animals that are less sentient?

We have opted to use mice as supposed to other less sentient species because there is a very extensive work already done with these animals that will facilitate our research. We need to use adult and ageing mice so that we can apply our findings to the human disease.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have implemented statistical process control charts to monitor for tumour growth and we will expand on what we delivered on our current project licence. In our current licence we also devised a better way of delivering drugs intravenously that we will continue using for the purpose of this licence. We will seek advice as we go along from our vet in order to continue to improve post-operative care and pain management.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 , ARRIVE guidelines (PLOS Biology, 2020), Guidelines for the welfare and use of animals in cancer research (BJC, 2010), RSPCA and LASA Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies (M. Jennings ed. 2015) and any NC3Rs guidance.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

I have signed up for the NC3Rs newsletters and I check their website regularly. I will also contact and communicate with people that have expertise and the Named People.

Home Office

NON-TECHNICAL SUMMARY

151.Production, Breeding & Cryopreservation of GA Mice

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (e) Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work

Key words

Transgenic, Cryopreservation, Chimera, Sperm, Embryo

Animal types	Life stages
Mice	juvenile, adult, embryo, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to provide a full transgenic service to researchers within the Institute and to a lesser degree the wider research community within the University if no alternative service is available.

The service will include

- Generation of new Genetically Altered (GA) lines by the most appropriate method. New mouse models will be transferred to the end user's PPL with authority to receive these mouse models for further use.
- Cryopreservation of GA lines by either sperm or embryo freezing.
- Rederivation of lines from either fresh or frozen embryos, or by In Vitro Fertilisation (IVF) from fresh or frozen sperm.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

- There is a continued demand for new animal models within the Institute and to have the expertise 'in-house' negates the need to import new GA mice from other suppliers.
- Cryopreservation of lines at an early stage in breeding programmes is required to reduce unnecessary breeding and allows users to rederive lines after several generations to reduce the risk of genetic drift.
- Onsite rederivation into a clean facility leads to a better health status, plus improved animal welfare through elimination of potentially harmful bacterial, viral and parasitic pathogens. This also has the beneficial effect of improving the validity of scientific data, as such harmful agents may adversely affect host physiology and therefore have a confounding effect on experimental results. Rederivation from frozen sperm and embryos also improves animal welfare by reducing the need to transport live animals between different research centres.

What outputs do you think you will see at the end of this project?

The purpose of this licence is to provide research scientists with a full transgenic service facility to include the production of Genetically Altered (GA) mouse lines, plus a cryopreservation and rederivation service.

Who or what will benefit from these outputs, and how?

This service will benefit the scientists of the Institute and to a lesser extent other scientists of the research community of the University.

How will you look to maximise the outputs of this work?

As a group we work closely together, we all regularly attend meetings where new and refined techniques are discussed, these are implemented within our work if we feel they will benefit the service that we provide in terms of NC3R's policies and efficiency.

Detailed record keeping is essential and ensures that any problems are identified at the earliest possible time and that output is monitored at every step.

Species and numbers of animals expected to be used

- Mice: 8000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use mice in this project as we are a transgenic core service that provides genetically altered mice for research groups in the Institute. Mouse is the species of choice for genetic modifications modelling human disease because of the availability and ease of manipulation of mouse embryonic stem cells.

The life stages of mice listed for this project reflects the fact that we expect to breed and maintain mice through all stages of the mouse life-cycle, from birth to adult. Genetically altered animals will be killed before they reach 12 months of age.

Normally females between the age of 3-5 weeks old will be superovulated for the production of embryos/oocytes for genetic modification, cryopreservation and rederivation. Females in this age bracket produce a higher yield of ova.

Typically, what will be done to an animal used in your project?

Approximately 50% of the total mice used in this project will be superovulated females which are then killed and their embryos/oocytes harvested.

Adult male mice from genetically altered lines may be used for breeding purposes, and then killed and their sperm harvested for cryopreservation.

A colony of wildtype adult females is maintained for use as embryo recipients. They are mated to genetically sterile males to induce a pseudopregnant state and then undergo either surgical or

nonsurgical embryo transfer. Once they have delivered and raised their pups to weaning age, the embryo recipient dam is killed.

Genetically altered animals that have been produced or rederived will be transferred to the end users PPL.

The majority of mice maintained on the Breeding and Maintenance protocol are killed and replaced by 8 months of age. In rare cases, we may keep a genetically altered mouse for up to 12 months of age if it is a stud male that demonstrates particularly good breeding performance, or it is of a particularly desirable / difficult to obtain genotype.

Where required, animals will be earmarked to obtain a sample for genotyping. This generally takes place when the animal is around 2 weeks old.

What are the expected impacts and/or adverse effects for the animals during your project?

Intraperitoneal and subcutaneous injections should involve only slight and transient pain.

Harmful adverse effects are rare and unpredictable in the production of new GM lines but any animal showing an unwanted harmful phenotype will be killed by schedule 1 method.

All mice that have undergone a laparotomy will have fully recovered within 24h, if not they will be killed by a schedule 1 method.

Ear notching should involve only slight and transient pain and no healing problems. If tail tipping is used then the pain will be controlled using a local anaesthetic, and any bleeding will be controlled by localised pressure.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

1. Superovulated mice; we expect 100% to be mild.

2. Founder mice; genetically altered animals produced by our service are not expected to exhibit a harmful phenotype. The majority of Chimeras are unlikely to display a harmful phenotype prior to weaning and are maintained under a sub-threshold severity until they are passed onto the end users PPL. Greater than 90% will have a sub-threshold actual severity.

3. Embryo recipient mice; approximately 70% of the recipients will be exposed to a mild severity as the embryos will be implanted non-surgically, the remaining embryos will be implanted surgically which has a moderate severity (30%).

4. Breeding and Maintenance of GA mice, we expect 80% to be returned sub-threshold and 20% returned mild.

What will happen to animals at the end of this project?

- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The justification for individual experiments will be covered in the end users licences. There is no substitute for a mammalian model. Mouse is the model of choice for genetic modifications modelling human diseases because of its availability and ease of manipulation of mouse embryonic stem cells.

Which non-animal alternatives did you consider for use in this project?

Zebrafish and lower vertebrates.

Although whole-animal models are necessary for understanding how human genes function in the context of normal development, much can still be achieved with cell lines.

Labs within the Institute use cell culture systems that mimic red blood cell production to generate and test hypotheses and new scientific methods.

We also ask researchers requiring a new GA line to test all constructs *in vitro* prior to requesting a GA mouse to be made.

On our request forms end users are asked to verify that they have used various search engines to see if they are able to find any non-animal alternatives.

Why were they not suitable?

Zebrafish and lower vertebrates may be appropriate for studying many developmental processes, but a mammalian model remains necessary to fully understand the effects of human genes and their disease associated mutants. This also applies to other *in vitro* systems.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of mice required has been estimated from the number used during the lifetime of the previous PPL. We anticipate that the requirement for the services provided by the Transgenic Group will steadily increase, but the number of mice used will remain constant as we become more efficient and new techniques are employed.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The use of CRISPR/Cas9 technology should reduce the numbers of mice used in the production of GM mice.

Breeding colonies to supply the service will be maintained at the lowest possible levels and any excess mice will be made available to other users.

Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Sperm freezing will be encouraged over embryo freezing as this will reduce the number of mice required to cryopreserve a line by eliminating the need to superovulate large numbers of female donors and maintaining stud males to produce fertilised embryos for freezing. Sperm can be frozen from males that would otherwise be killed.

All steps in every process will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers.

Animals are promptly sampled for genotyping so that any animals which are of the incorrect genotype are not kept within the colony. End users must demonstrate that the genotyping protocol is validated and robust before the generation of a new mouse model.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

One of the aims of this project is to supply GA mice to end users who have the authority to use them on their own project licence. Mouse is the species of choice for genetic modifications modelling human disease because of the availability and ease of manipulating mouse embryonic stem cells.

As a transgenic core service we have the ability to provide these mice in an efficient way that minimises the number of mice used, the pain, suffering, distress or lasting harm that those mice could experience in achieving the end product.

For example, where the administration of injectable substances is required for superovulation or the provision of analgesia, the most appropriate administration route and dosing regime is used to achieve the required outcome.

In addition, non-surgical embryo transfer methods are used to achieve pregnancy in recipient females wherever possible. However, in approximately 30% of cases, surgical embryo transfer must be performed for scientific reasons relating to the stage of the embryonic development and where the embryos have to be placed within the reproductive tract. Our protocols for performing surgical embryo transfer have been carefully refined in order to reduce the amount of pain and distress experienced by recipient females to the absolute minimum.

Why can’t you use animals that are less sentient?

The aim of this project is to provide end users with GM mice, the justification for which will depend on their own scientific goals. Typically, the justification for using mice is that lower order organisms do not exhibit the required features. Flies for example, do not have an adaptive immune system.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Where substances are administered by injection the minimum effective dose and the most refined route will be chosen.

Wherever possible constructs and/or manipulated embryonic stem cells will be produced and tested in the bioengineering facility in an *in vitro* system before going on to produce new GM lines.

Non-surgical embryo transfer will be the preferred technique opposed to surgical transfer, depending on the stage of the embryo.

All surgery will be performed aseptically and analgesia used wherever appropriate.

Whilst this service licence includes the option for cryopreservation of embryos, we will always recommend sperm freezing as the most refined methods of cryopreservation before we agree to carry out embryo cryopreservation.

Through the use of efficient breeding and colony management we can reduce the number of mice that are being culled surplus. Through our management process animals are generally retired from breeding by 8 months of age, this reduces any potential for breeding related complications.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All surgery will be done aseptically according to guidelines e.g. LASA Guiding Principles for Preparing and undertaking Aseptic Surgery. http://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgeryfinal.pdf NC3R's.

Home Office efficient breeding of Genetically Altered mice.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We ensure effective breeding of colonies in line with the principles of the 3R's. Advancements will be identified through the regular attendance of conferences such as LASA, ISTT and the NC3R's technical symposium. New methodologies and procedures will be implemented as they arise.

Home Office

NON-TECHNICAL SUMMARY

152.Protecting and repairing injured retinal ganglion cells

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Neuroscience, optic nerve injury, neuroprotection, axon regeneration, loss of vision

Animal types	Life stages
Mice	adult, juvenile, neonate, pregnant
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand the factors that cause death of retinal ganglion neurons and prevent their nerves from regrowing after injury, with a view to developing therapeutic agents to counteract this and ultimately preserve visual function.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Traumatic optic nerve injury affects 2-5% of people worldwide with 0.5-5% also occurring in patients with head injuries, whilst 60 million people are affected by glaucoma, leading to loss of vision and eventual blindness. All of these conditions cause death of retinal ganglion cells, neurons that form the optic nerve and relay visual information to the brain. Currently there are no therapeutic agents that promote neuroprotection and axon regeneration after injury or disease to the optic nerve and hence blindness results. We wish to identify and test effective therapeutic targets and agents that will protect neurons, particularly retinal ganglion cells, from death and promote their nerves to regenerate after injury, ultimately protecting against visual function loss.

What outputs do you think you will see at the end of this project?

The primary outputs of this work will be the information on identifying new therapeutic targets to prevent the death of retinal neurons and strategies to promote their axon regeneration after injury. A major potential benefit from the work proposed will be the acquisition of new knowledge for dissemination in peer-reviewed journals. Functional outcomes after ocular damage are poor at best and presently only palliative drug treatments are available for patients. There is, therefore, an unmet clinical need for new therapies aimed at enhancing functional repair responses of damaged ocular tissues.

Specific academic outputs will be to publish the findings. This is important for the development of the project but also to provide a knowledge base for others working in this field.

Other product outputs will be to support the development of the intellectual property already filed and allow it to translate it into a commercially viable proposition for the establishment.

Who or what will benefit from these outputs, and how?

In the short term the benefits would be to provide high impact publications and work for the researchers involved and in raising the profile of early career researchers. This work will also potentially support an application to the MHRA for a clinical trial of the DNA damage inhibitors in eye injury and disease models.

In the longer term this work has the potential to have an immense impact on the lives of patients with eye injuries and diseases where death of the RGC and its nerves lead to blindness. There is currently no treatment that reverses the pathological effects of optic nerve injury/disease and the functional loss that occurs as a result. Our DNA damage inhibitors for example, would provide a clinically viable small molecule-based treatment that can potentially reverse the effects on injury and prevent blindness.

Examples of other potential beneficiaries of the success of this work are glaucoma patients who will benefit through the development of new treatments that are neuroprotective, pro-regenerative and antiscarring, since the same pathological processes occur in this group of patients. At present, no treatments are available to combat neural tissue loss/dysfunction or fibrotic diseases of the eye and this is entirely attributable to our ignorance about the injury and disease processes. Additional beneficiaries could include health care providers, particularly the NHS.

Currently the NHS spends around £25billion/year on patients with partial blindness that includes eye injuries and glaucoma. Effective therapies that protect RGC from death and promote their nerves to regenerate, if successful, would relieve the pressure on the NHS and significantly reduce the cost burden of treating visually impaired patients.

How will you look to maximise the outputs of this work?

We will maximise the outputs of the work by collaborating with academics and companies working in this field to maximise the use of the data we obtain. We will rapidly disseminate the outcomes of the tests whether, negative or positive to inform the community and support other researchers developing technologies in this area. We are already working with several small and large enterprises which we will seek to attract after proof-of-principle experiments have been successful. Some of the agents including the DNA damage inhibitors will need to be provided by companies collaborating with us, however, we are currently filing re-purposing patents for use in our disease models and hence there should be no problems with the freedom to publish our results and without restrictions. However, many companies require vetting of each publication prior to submission, but we will ensure this is completely in a timely manner.

Species and numbers of animals expected to be used

- Rats: 1500
- Mice: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Typically, rats are used for most of our experiments since they closely mimic the human molecular responses to optic nerve injury. Although mice do not faithfully reproducible the molecular characteristics of injury as well as rats, mice do offer the opportunity to assess our objectives in knockout animals, where the impact of a particular gene to aspects of neuroprotection, axon regeneration and scarring after optic nerve injury can investigated.

Typically, what will be done to an animal used in your project?

Typically, animals will undergo unilateral optic nerve crush injury followed by intravitreal injections of therapeutic agents for up to 6 weeks. The thickness of the retina will then be quantified using non-invasive optic coherence tomography followed by electroretinography to determine retinal function. Animals will then be killed and tissue collected for histological/protein/mRNA analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Optic nerve injury: This cause blindness in one eye since the optic nerve is injured (100% of animals). However, since rodents do not rely on vision as a primary sense, this does not impair movement, behaviour, feeding or drinking as measured by clinical signs.

Substances administered by injection: Stress due to restraint and transient discomfort from needle insertion

is likely in 100% of animals. These are minimised by selection of appropriate (minimum possible) sized sterile needles and syringes.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All animals in this project licence will experience a maximum severity of moderate.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Whilst some elements of optic nerve injury can be modelled in cell culture, the complex, clinical picture and interaction of the whole-body systems, including in particular the immune and nervous systems cannot all be currently modelled in cell-culture or computer-based models. The use of live animals is therefore unavoidable and essential for drug discovery and to demonstrate the activity of drugs in a situation relevant to human disease. Neurons are not present outside the animal kingdom and so an animal is required. Only mammals have a sufficiently developed immune-system to readily compare to humans, and rodents are the animals of lowest neurophysiological-sensitivity required to achieve the scientific aims. It is not ethical to conduct experiments on humans in multiple sclerosis, especially where those experiments require the removal of parts of the immune or nervous system for ex-vivo investigations. Therefore, there is no feasible alternative that can entirely replace the use of a living animal that would allow the objectives to be met. However, we will use in vitro and ex vivo work prior to or in parallel with animal studies.

Which non-animal alternatives did you consider for use in this project?

There are currently no alternatives to animal work for the optic nerve/retinal injury model.
No cell culture-based models exist that encompass all of the aspects of disease for any of the models described in this project. However, individual aspects will be modelled in vitro and ex vivo. For example, we regularly use in vitro retinal cultures to detect therapeutically useful molecules in terms of neuroprotection and axon regeneration to then take forward into animal studies.
We also use ex vivo whole eyes to determined drug penetration through the barriers in the eye after topical application.

Why were they not suitable?

The fundamental reason why the use of animals is required is to understand these processes that at present no in vitro methods can model the complexities of the systems involved in this disease. It is difficult to use primary cells to culture all of the different types of cells since they require different growth mediums and factors for survival. Indeed, the reason why many new drugs fail between cell culture and in vivo studies is in the inability to full recapitulate the in vivo environment. Technologies are being developed to address this gap, including the development of 3D cultures. However, none of these model systems are yet able to phenocopy the integration

and interplay between the numerous cell types that constitute optic nerve/retinal injury.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers of animals have been based on pilot data, in-house data and published data where our own data is not available.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We used the NC3Rs EDA system to calculate animal numbers to be used for this project. We used values from several of our own and other's published data to facilitate power calculations and reduce animal usage.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will seek to refine protocols, such as the development of novel quantitative outcome measure that will facilitate “reduction”. Experiments will be planned so they can be published in accordance with the NC3R’s ARRIVE guidelines.

Wherever possible, we will use archived samples to avoid repeating controls and sham treated groups.
As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material; and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated). We will make appropriate arrangements to randomly assign animals to experimental groups and blind studies.
At the end of the experiment we will harvest the maximal possible number of tissues. Tissues not immediately analysed will be archived and will be made available to other researchers working on similar questions.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Optic nerve injury exhibits many pathological processes including neuronal death and axonal degeneration. Our mouse/rat models have proved to faithfully reproduce human optic nerve injury and have allowed drugs to be screened reliably. Optic nerve injury will be induced by a crush injury. This is very well tolerated and with high reproducibility, limiting the need for large group sizes and many repetitions of experiments. We aim to continue to refine these models and as we apply additional outcome measures such as non-invasive optic coherence tomography, we increase power to detect drug effects and enhance the utility of the models. Through the use of a reproducible system and defined endpoints for each objective, we can limit the time in procedure and as a result the suffering that the animal will accumulate as a product of injury itself.

Why can't you use animals that are less sentient?

We cannot use less sentient species (e.g. zebrafish) for this work, because unlike mammals, most are able to regenerate their CNS spontaneously. Rats are typically used for our experiments since they share similar pathophysiology to humans after injury to the CNS. Mice are also used since there is established and reliable transgene technology, and established models of optic nerve injury. There are a large number of genetically modified mutants available and there is extensive amount of work that has already been performed and published using mouse and rat models of optic nerve injury.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All therapeutic agents are evaluated and optimised in vitro prior to in vivo application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are done on harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. Small numbers of animals (i.e. 6-8 rats/mice) are used in the optic nerve crush model to maximise the effectiveness of our post-disease care. These refinement steps significantly reduce animal usage and severity.

Also, we have switched to unilateral eye optic nerve crush experiments to avoid blinding animals in both eyes, avoid loss of normal blood perfusion to the eye by avoiding crushing of the retinal artery that runs parallel to the nerve and is susceptible to crushing and by moving extraocular tissues to one side rather than removing them from the orbit completely. All of these steps minimises stress and potential wastage of animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment.

Experiments will be conducted in accordance with the guidelines published by the Laboratory Animal Science Association (LASA).

The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed by advances in the 3Rs through attendance of seminars and conferences, as well as discussions with the NVS and NACWOs and NIOs.

We will review each experiment on completion to determine any refinements that can be applied to future experiments.

Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.

We will also stay up to date with guidance published by FELASA on the most refined experimental methods.

Home Office

NON-TECHNICAL SUMMARY

153. Protection against incapacitating agents

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

therapy, incapacitating agents

Animal types	Life stages
Marmosets	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Pharmaceutical based agents that can cause incapacitation pose a threat to the UK. This project aims to understand the threat posed by such agents and to assess potential treatments for them.
A retrospective assessment of these aims will be due by 09 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This licence will help to understand the threat posed to the UK from pharmaceutical based agents deployed either as incapacitating agents or as lethal agents. The data from these studies will aid in determining the level of threat posed and the requirements for detectors, diagnostics, physical protection, medical countermeasures, extended medical management and decontamination.
If an agent is determined to be a threat, then existing or novel medical countermeasure will be tested to assess their ability to reverse, prevent, reduce, delay or mitigate the effects of exposure. Effective medical countermeasures will be able to save lives.
These agents pose a potential threat to the civilian population, first responders, the NHS and UK Armed Forces.

What outputs do you think you will see at the end of this project?

There will be improved knowledge on the potential threat posed by pharmaceutical based agents as either incapacitating agents or lethal agents. This knowledge will aid in determining the level of threat posed and the requirements for detectors, diagnostics, physical protection, diagnostics, medical countermeasures, extended medical management and decontamination.
There will be improved knowledge on the effectiveness of new and existing medical countermeasures to reverse, prevent or mitigate the effects of exposure to pharmaceutical based agents.

Who or what will benefit from these outputs, and how?

Increased knowledge of the threat and new medical countermeasures will provide benefit to the civilian population, first responders, UK armed forces and our international partners (nations with whom the UK formally collaborates).
Outputs from this work will aid in the development of new detectors, diagnostics, physical protection and decontamination strategies for pharmaceutical based agents.

How will you look to maximise the outputs of this work?

Some of the work will be published in the public domain in peer reviewed journals.
Outputs not suitable for the public domain will be shared with international partners through formal international collaborations. These comprise regular prearranged collaboration meetings / conferences.
Where the long term output of this work is a variation to the currently licensed indications for use of a therapy (for example to add that the therapy be used in a new way to treat exposure to a pharmaceutical based agent) this will be disseminated via medicinal product information and will be accessible to healthcare professionals.

Species and numbers of animals expected to be used

- Marmosets: 358

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using adult marmosets as we know that they have a similar response to man for the compounds we will be studying.
We also know that other species such as mouse, rat, rabbit and pig respond differently to these compounds and / or are a poor predictor of the human response.

Typically, what will be done to an animal used in your project?

Most animals will receive an inhalation exposure to a pharmaceutical based agent (PBA). This will require they are restrained for exposure which also enables the measurement of respiration during the exposure. Exposure to the PBA will likely cause a range of effects such as: sedation, anaesthesia and respiratory depression. This respiratory depression may become fatal. Animals will be observed following exposure until they recover. Some animals may be exposed to PBA via a different route, including injection.
Some animals may receive potential medical countermeasures to assess their ability to block or reverse the effects of PBA exposure. Medical countermeasures may be administered before or after PBA exposure and will be administered by injection. Some animals may be surgically implanted with a drug delivery device containing medical countermeasures. Some animals may be implanted with a device that enables access to blood vessels via a sampling / injection port.
Animals will have blood samples taken to measure the concentration of PBA and / or medical countermeasures in the blood.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals exposed to PBAs are expected to experience effects such as drowsiness, sedation, anaesthesia and respiratory depression. This respiratory depression may result in death, however, the animal will be anaesthetised by the PBA if this happens. Animals that survive will make a complete recovery. The effects are expected to last for hours.
Medical countermeasures are likely to fully or partially block or reverse the effects of exposure to the PBA.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The severity limit of all protocols is moderate.
It is expected that across the protocols, 56% of the animals will reach moderate severity with the remaining 44% reaching mild severity.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 09 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Exposure to fentanyls can cause a complex range of effects with respiratory, cardiovascular and neurological changes. It is important to understand the doses required to cause a range of incapacitating effects and to cause death as this provides crucial information on the threat posed. It is also important to understand the window of opportunity in which to treat exposure and how long the effects may last. Such data cannot currently be gained without the use of animals to model the whole body response to fentanyls.
Some data can be gained using *in vitro* assays, such a opioid receptor binding potency, however, whilst fentanyl effects are driven by activity at the opioid receptor, fentanyls also affect different signalling pathways. The efficacy of nAChR partial agonists in a rodent model of opioid induced respiratory depression shows that opioid induced respiratory depression and its mitigation involves complex signalling within the central nervous system. Therefore it is important to understand the effects of exposure and countermeasures in an animal model.
In vitro systems can be used to determine if opioid antagonists are able to displace fentanyls from opioid receptors, however information is still required to determine the length and extent of protection that can be afforded, whether planned doses of opioid antagonists are able to counteract predicted challenge levels and whether they can treat all effects of exposure.

Which non-animal alternatives did you consider for use in this project?

We have considered in vitro systems and simple model organisms for this project.

Why were they not suitable?

Such approaches can provide useful data, however, they will not be able to achieve aims of our project and would not provide accurate predictions to man which will focus on one class of chemical agent. However, supporting information on novel compounds will be potentially be provided by in vitro systems, simple model organisms and other non-primate animals.
A retrospective assessment of replacement will be due by 09 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These numbers are based upon work that is currently planned and group sizes that have been used successfully in previous studies in this area.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Advice has and will be taken regarding study design from statisticians with respect to statistical designs of studies and the analysis of data from this project. Advice has and will be taken from pharmacokinetics experts regarding design of kinetics studies.
If multiple treatments are to be tested, then a shared control group will be used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Before treatments are tested, a pilot study will be carried out to assess the selected challenge dose to determine its variability and suitability.
If multiple treatments are to be tested, then a shared control group will be used.
A retrospective assessment of reduction will be due by 09 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Animals will be exposed to pharmaceutical based agents which will themselves cause analgesia, sedation and anaesthesia and will reduce the potential pain, suffering and distress that the animals may experience. Any adverse effects from the exposure will occur once the animal has become anaesthetised by the exposure. If treatments against exposure are effective, then this will prevent or reduce the effects of the exposure.
Some animals may undergo surgical procedures to either allow monitoring of physiological parameters or to administer treatments over an extended period of time. These animals will be given analgesics during their recovery from surgery.

Why can’t you use animals that are less sentient?

The project is to understand the potential threat from pharmaceutical based agents, many of which have an anaesthetic action. Therefore, it is not possible to do the study in terminally anaesthetised animals.
Lower species have been shown to be a poor predictor of human response for the class of pharmaceutical based agents that will be initially studied.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be continually monitored following exposure until they have made a full recovery or reached a humane endpoint.
For animals undergoing any surgical procedure, appropriate analgesics will be given pre- and / or post-surgery. Animals will be monitored as they recover from anaesthesia and will be regularly checked post-surgery to ensure appropriate level of analgesia and monitor the progression of wound recovery. Sub-cuticular stitching will be used wherever possible to minimise any discomfort due to stitching.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Available guidelines on the NC3Rs website regarding blood sampling will be followed. This references "A Good Practice Guide to the administration of substances and removal of blood, including routes and volumes" by Diehl et. al. This will also be followed regarding the administration of substances.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Updates on advances in the 3Rs are regularly distributed by our Named Information Officer. The licence holder is actively engaged in our establishments Animal Welfare and Ethical Review Body (AWERB). Any appropriate advances will be discussed with our veterinary staff and, where appropriate and compatible with the scientific aims of the project, these advances will be incorporated. I will maintain my required annual CPD, attend relevant external scientific meetings and have meetings and teleconferences with international collaborators working within this field. **A retrospective assessment of refinement will be due by 09 January 2026**
The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

154. Protein kinases and ion transporters in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Kinase, Ion transporters, Cellular chloride volume regulation, Kinase inhibitor, Therapy

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This proposal will elucidate the role of a key enzyme in cellular chloride volume regulation. We will use genetic

models of mice and a drug that specifically target this pathway to aid the study.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Chloride is a very important ion in the brain, because nerves use it to give negative signals in synaptic inhibition, an 'off switch' for nerves. For this to work, however, chloride needs to be kept very low inside nerves. On occasions, nerves lose the ability to remove chloride, and when this happens, this synaptic inhibition no longer works well. This can present serious problems for brain function, and may give rise to seizures, spasticity, severe pain and multiple other neurological conditions, such as epilepsy, stroke and autism etc.

Despite the clear importance of maintaining a good chloride balance, we know little about how this is achieved in healthy brains, or why it might go wrong. Epilepsy is a family of diseases, defined as the experience two or more seizures occurring more than 24 hours apart with no immediately identifiable cause. Epilepsy is common (>50 million people with epilepsy worldwide, and >600,000 in the UK), casting a huge socio-economic impact because it is often a lifelong condition. Moreover, about 30% of people with epilepsy remain refractory to currently available medication, meaning that there is a strong imperative for developing novel therapies.

We discovered a malfunction in the way key proteins are transported within the brain after epilepsy leads to excess chloride levels and swelling, which can cause severe damage. When these proteins are activated after epilepsy, it is just like a water tap is being switched on, and out of control, thus the sink is overfilled with tap water. So the key is to find a switch that can control the tap. We then identified a pathway could directly control the activity of these proteins which bring too much chloride into the nerves, and this pathway is like the "switch" for the tap. This 'switch' is a drug target, for the treatment of epilepsy.

We have developed and patented a new compound that specifically targets this pathway. Our tests in cultured cells have shown that the compound may be able to stop cell swelling (increased chloride volume). This drug effect is unlike any other anti-epileptic medication on the market, so represents an entirely new way of treating the condition. The drug is still in the laboratory and requires further development. So far it shows promise in effectively reducing brain swelling in conditions of stroke or epilepsy. We expect another 3-5 years' work to optimize the drug properties, before it gets ready for a clinical trial.

We have developed models of mice in which certain parts of the pathway are altered to prevent other proteins' actions. The mouse models and new drug will now allow us to perform many experiments to extend our understanding of this important pathway. And crucially, these will also allow us to test the drug, and other related drugs as they are developed. In particular, we will provide the first tests of how this pathway is involved in epilepsy, using several different mouse models, and also test whether the drug can make the condition better. This are critical studies in the steps towards designing and performing clinical trials of these new drugs.

It is these questions that we intend to explore in this project license.

What outputs do you think you will see at the end of this project?

This project will provide a robust framework of knowledge of how these pathways operate and regulate chloride levels in cells. The new compound we have developed has good potential for the treatment of epilepsy. This information is crucially important for the development of effective drugs in this area. Research data will be presented in scientific conferences, and published in high impact journals.

Who or what will benefit from these outputs, and how?

Our research has the potential to lead to the development of a new treatment for epilepsy, a potentially life-altering complication of epilepsy that currently has only limited and expensive therapeutic options. Thus, this

work may have a positive impact on a number of important beneficiaries expanded on below.
Briefly: (1) Patients with epilepsy or high blood pressure; (2) Healthcare professionals, the NHS and the Pharmaceutical industry; (3) Wider scientific community (4) Researcher working on the project; (5) Society; (6) Public engagement; (7) Local economy.

(1) Importantly, in the longer term, this work should benefit people with epilepsy and their families. Epilepsy is a disorder of the central nervous system characterized by recurrent seizures unprovoked by an acute systemic or neurologic insult and has significant impact on the quality of life and psychological welfare of sufferers and their families.
A new therapy to halt the progression of epileptogenesis could lead to the therapeutic use of these drugs in other conditions.
(2) Healthcare professionals including physicians and epilepsy specialist nurses will benefit from increased patient satisfaction and greater patient confidence since current therapies for epilepsy are limited. Epilepsy may require chronic treatment with antiepileptic medication and, in some cases, surgery. A major drain on resources is that many epilepsies remain refractory to the available medications. New therapies, to minimise epilepsy-associated damage and prevent life changing disabilities inflicted by epilepsy, are desperately required.

(3) The wider scientific community will benefit. This project will improve our understanding of the mechanisms of how chloride level is regulated in cortical networks, and through advancement of methods used to study this expanding field of research. We make our results and methods freely available to national and international collaborators in a diverse range of projects. In the long term, this project will aid our understanding of epilepsy and may highlight new pathways for therapeutic intervention.

(5) Society. With an estimated incidence of 34 to 76 new cases per year per 100,000 people, epilepsy affects about 70 million people worldwide. This represents a significant cost to society in terms of time off work as the disease progresses, disability allowance, caring for individuals with epilepsy, i.e.
healthcare services and family if they need to give up work to become carers. Additionally, sufferers can experience depression and anxiety and the cost of treating these associated conditions would be reduced.

(6) This project will have a positive impact on public engagement with science. The potential to develop a new therapy for a debilitating and feared disease will engage public interest and raise awareness of the researchers themselves, their academic institutions and the MRC.

How will you look to maximise the outputs of this work?

Scientific Publications:
Primary research results will be published in high impact journals that are widely circulated. The research findings will initially be published in preprint servers, e.g., BioRxiv, then they will be submitted to a high impact peer reviewed journal. Particular emphasis will be placed on publishing the work as Open Access in order to maximise access and benefit from the work. We anticipate one major publication will be generated from this work. Given the impact of the discovery, we anticipate submitting the work to Science or Cell, and if unsuccessful with these we will then submit the work of Molecular Cell or EMBO J.
In addition to primary research articles, reviews, commentaries, and highlights will be published to summarise and promote the research findings. These types of publications often have a different kind of audience to those of primary research articles. Thus, this will make it possible to reach an even bigger audience.
E-communications, Press releases and Media:
Press releases from the involved labs on the proposed research will be highlighted in news items on University webpages. These are often circulated to the Universities' staff and students to maximise exposure. These sites will be regularly updated with any publications from this work that describe exciting discoveries, so that their combined efforts in highlighting our work can make it reach wider audiences and therefore maximise the impact

of the research. A great emphasis in communicating the outputs of this project will be placed on social media. University, and the Medical School social media channels, Twitter and Instagram, will be used to communicate the key findings of this project. Additionally, the investigators' Twitter, LinkedIn, ResearchGate, Scopus and Instagram will also be used to populate this project's research findings. A pdf copy of the Open Access papers generated from this work will be placed in various platforms such as ResearchGate to maximise access to our published work. For the social media outlets, purposefully produced figures aimed at grabbing the attention of the readers will be designed. This approach has already been adopted in disseminating his recent publications on social media and it led to significant viewing figures. For instance, our recent Nature Communications paper with the discovery of the novel drug was reported in India, USA, UK, Germany, China, South Korea, Singapore and Australia through 47 different articles/broadcasts. This gave a potential reach of 16,396,000 (based on how many people view the website each day and could potentially have read the article on the research) within the first week of the publication.
Oral and Poster Presentations:
The Principal Investigator will actively encourage the researchers involved with the work to present the research results in national and international meetings in every possible occasion. We envisage sending the researchers working on this project to at least one national/international meeting per year.
In particular, the EMBO meetings as well as meetings and conferences organised by the Epilepsy Society UK, Biochemical Society and the British Pharmacological Society, which are always well organised and attract excellent speakers. Also, such meetings often cover research topics that are of interest to the project investigators and the proposed work.

Species and numbers of animals expected to be used

- Mice: 2500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

According our previous studies, most of the mouse models we intend to use, do not exhibit any overt adverse effects. Some mouse models could manifest with high blood pressure,
We will use genetically altered mouse models and drug treatments for our studies. We will generate cell lines from mouse embryos or pups (postnatal day 0-7) for most of our studies. For some studies we will need tissues taken from adult mouse brains to see if our drug is effective for the treatment of epilepsy *in vitro*. For our final studies we will need to use adult mice treated with our drug.

Typically, what will be done to an animal used in your project?

For generation of cell lines, mouse embryos, mouse pups or adult mice will be humanely killed and tissues harvested for *in vitro* studies. .
In some studies, animals will receive injections of our drugs and will then be humanely killed and tissues taken for *in vitro* study.

What are the expected impacts and/or adverse effects for the animals during your project?

General breeding.

Genetic alteration does not cause any overt adverse effects for these two genetic modified models of mice. They have normal phenotype and lifespan. According to our breeding experience on these models of mice, they did not cause any short term mild pain, suffering or distress on the basis of the welfare assessment performed on the established lines.

Potential but unlikely adverse effects:

Most animals produced under this protocol are not expected to exhibit any harmful phenotype but are expected to experience mild levels of severity. Signs of adverse effects are described below.
Some animals may have the potential to develop a harmful phenotype, e.g. tumours, neurological signs, after a certain age but in all cases we anticipate animals will be killed before reaching that age and before the onset of clinical signs. . Animals exhibiting any unexpected harmful effects will be humanely killed

Pharmacological administration

Potential or unexpected adverse effects:

During pharmacological administration, there may be some unexpected signs, e.g. drop of body temperature, hunching, piloerection, loss of righting after the drug administration. Unexpected effects of injection site irritation, such as erythema, itching, discomfort and pain, including more severe manifestations such as ulceration or necrosis, may be seen very occasionally after the drug administration.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

It is 'mild' for all studies.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of animals will be necessary for the success of the research programme because mouse genetics is one of the most powerful technologies to examine the physiological roles of proteins in health and disease. Animals will be required for two major purposes:

1) To generate cells for our cell culture systems.

Cell culture will be our main model system for studying these pathways in the nervous system. Cells taken from embryos or very young mice are the preferred choice for these purposes. We have found that our culture systems are good predictors of the effects seen *in vivo*. These cultured cells are short lived and therefore must

be generated on a regular basis for our experiments.

2) To test the effects of our compounds on the development of epilepsy in whole animals.

In the second phase of the research programme, it will be imperative to extend our observations made using cell culture systems into living animals and test new strategies for the treatment of epilepsy. A neural circuit is a population of neurons interconnected by synapses to carry out a specific function when activated. Neural circuits interconnect to one another to form large scale brain networks. Unfortunately, it remains impossible to reconstitute this complex pathological response in cell culture systems. Thus, we need mouse brain slices for *ex vivo* studies, to see the drug effect for the treatment of epilepsy.

Which non-animal alternatives did you consider for use in this project?

Cell lines, Non-regulated species: for examples *Caenorhabditis elegans* or *Drosophila*, the use of early developmental stages of protected animals, functional magnetic resonance imaging (fMRI) in human patients, and computer modelling etc.

Why were they not suitable?

I have studied information from the SyRF website and Fund for the Replacement of Animals in Medical Experiments (FRAME) website for alternatives to the use of animals for these studies. There are some possible alternatives, however, they are not appropriate for use in this study:

- Cell lines: Cell lines will be used to validate the molecular activity/specificity of any pharmacologic agents *in vitro* before they are used in animals reducing the number of animals used.

- Non-regulated species: *Caenorhabditis elegans* or *Drosophila* for examples, have simple nervous systems with synaptic circuits. However, these species do not have the level of neuronal network complexity observed in mammalian species and often do not have the same or even analogous molecular machinery associated with neuronal function. In addition, the types of complex behaviours associated with diseases of cognition cannot be easily modelled in these species.

- The use of early developmental stages of protected animals: As the focus of this work is on the effects of epilepsy and developmental brain, both prenatal and postnatal developmental stages are required. In this case, it is impossible to use only earlier stages of development.

- Functional magnetic resonance imaging (fMRI) in human patients: fMRI can provide useful clinical information regarding activation of certain brain areas whilst patients are performing certain cognitive tests. However, fMRI effectively records changes in blood flow to brain area, which is a surrogate marker for neuronal activity, and it is thus open to interpretive errors.

- Computer modelling: These approaches rely on high quality experimental data to inform and test the predictions of the models. Furthermore, due to the highly complex systemic nature of epilepsy, it is impossible to model with a computer (*in silico*) due to the infinite number of variables both known and unknown in the neuronal networks found in the mammalian brain.

During the course of this project, I will undertake regular reviews of the literature to keep abreast of *in vitro* techniques and approaches that could be used to replace animal use for the context of this project.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals used is based on our estimate of the minimal number that allows for sound statistical testing.
We have taken advice from statisticians, plus taking into account the variabilities according to previous experience and current pilot data, we calculate a total number of mice required to be 2500. 2000 mice are required for breeding and generation of cell cultures. This is calculated on 4 lines likely to be used over the course of the project. This agrees with our previous experience for the average number required for maintaining lines, and generation of primary cultures. A further 500 mice are required for use in Pharmacological Investigations. These numbers are in agreement with previous animal projects that I have been involved in.
Once specific research programmes come to an end, archiving of the line via cryopreservation of embryos or sperm will be used and the breeding stopped. We intend to breed and maintain GA mice in accordance with the ASRU GAA framework. We will continually measure production and breeding performance and ensure the minimum numbers of animals are used in the program.
The science outputs (i.e. research papers) from this project will be published in international peer viewed journals that support the ARRIVE guidelines.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

All sample numbers for mice experiments are based on calculations made using statistical software, for example: http://powerandsample.com and https://www.nc3rs.org.uk/experimental-design-assistant-eda. In our prior studies, we consistently found very large effects in every case, indicative of the importance of the pathway, and clear results using our transgenic models. For most of our studies we have found that we can generate results with fewer than 6 animals per group. In this proposal, we plan to use sample sizes of n=7 to include both adequate numbers of both male and female mice. If we observe trends indicating that gender has an effect in our model we will stratify the groups by gender for analysis. We believe that our study will be able to detect any differences between genders in our models of mice. As experiments may be started over a period of days a randomised block design will be implemented, whereby animals are randomised to treatment type and order of dosing on any given day. This will reduce experimental variability, and thus animal numbers. When possible, collaborations with experts in the field will be sought to ensure that experiments are performed as efficiently as possible.
Additionally to the sources above, statistical advice will be sought through the University Statistics Helpdesk.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

All *in vivo* and *ex vivo* experiments will be carried out in as controlled a manner as possible to reduce the animal-to-animal variability and thus reduce the number of animals used. Variable controls include:

- Wildtype littermate control bred under the same conditions and transgenic models will be used as a control comparison wherever possible.

- Age range of animals for a particular study minimised.
- Gender ideally kept the same. Where it is not possible or impractical to use a single gender for a study, a careful gender balance will be maintained and appropriate statistical analysis carried out.
- Environmental disruption (e.g. noise, light changes etc.) minimised (for *in vivo* experiments).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This proposal outlines proof-of-principle studies that will elucidate the role of a specific pathway in epileptic pathophysiology. We will use cellular assays of neuronal function to extend our knowledge of this pathway, and ultimately investigate its involvement in epileptic pathology, by testing the effects of a newly developed drug and its modified analogues in several different models of epileptic activity ***ex vivo***. We will utilise a range of transgenic animals, we designed previously, to interfere with the pathway at different sites; these provide powerful tests of our hypotheses about the pathway's role in disease.
All GA mice included in this project plan have already been established through targeting strategy.
Genetic alteration did not cause any overt adverse effects for these genetic modified models of mice. They have normal phenotype and lifespan. According to our breeding experience on these mouse models of mice, they did not cause any short term mild pain, suffering or distress on the basis of the welfare assessment performed on the established lines.
In particular, some mice have lower activity of a protein in the kidney, which would prevent animals from suffering high blood pressure according to our recently study. We expect these mouse models to be protected from developing epilepsy. With these mouse models, we will generate primary neuronal culture from **their earlier life stage**s, for cellular based assays. Adult mice will be humanely **killed**, and brain slices will be obtained for ***ex vivo* studies**, to see the drug effect for the treatment of epilepsy.
Our compounds and its modified analogues, will block the same pathway, mimicking the genetic effects. We will administer our compounds to adult mice to investigate drug efficacy. Compounds will be administered via Intraperitoneal (i.p.) injection at doses that are not expected to cause adverse welfare, on mice.

Why can't you use animals that are less sentient?

We need to use mammalian animals with a certain degree of neuronal complexity in order to achieve our scientific outputs. We use animals at an immature life stage where possible, for example, embryos or mouse pups for making cell cultures. Induction of epileptiform activity will only be conducted in brain slices taken from adult mice (*ex vivo*).

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Minimising animal suffering: We have several measures in place in order to reduce animal suffering, such as daily monitoring, extra bedding, and a welfare scoring system.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I have been following published practice guidance, for examples, "Guidance on the Operation of the Animals (Scientific Procedures) Act 1986" and "Guiding principles on good practice for Animal Welfare and Ethical Review Bodies, 3rd Edition – September 2015" to ensure experiments are conducted in the most refined way.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I follow the NC3Rs twitter, and I have subscribed to NC3Rs newsletter, from which I get the latest articles, events and funding opportunities from NC3Rs in my email inbox every month. I will also engage with our local NC3Rs representative.
I will also regularly attend appropriate courses to keep my ASPA knowledge up to date, such as ASPA refresher course, Sch1 training courses and the 3Rs Symposium.

Home Office

NON-TECHNICAL SUMMARY

155. Radiation Combinations for Cancer Treatment

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Radiation, Oncology, Rodent

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To test anti-cancer agents in combination with ionising radiation with the aim of improving current clinical therapies.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Radiotherapy provides significant benefit and is used in over 50% of all cancers. To combine radiotherapy with novel anti-cancer agents may significantly enhance the therapeutic outcome and has the potential to translate into highly significant clinical benefit.
In order to determine potential clinical benefit we need to have pre-clinical animal models that will demonstrate and characterise improvements in anti-tumour activity and relevant potential clinical benefits thus directing clinical development.

What outputs do you think you will see at the end of this project?

Data to support clinical trials. Improved radiation protocols combined with novel anti-cancer agents to be used in the clinic leading to improved efficacy and tolerability. We will share pre-clinical data with principal investigators to influence clinical trial designs. Publication of both successful and unsuccessful data in high impact journals.

Who or what will benefit from these outputs, and how?

Radiotherapy provides significant benefit being used in over 50% of all cancers. As the patient population treated with radiotherapy is so large, enhancing therapeutic outcome for even a relatively small proportion has the potential to translate into highly significant clinical benefit. To have pre-clinical animal models that will demonstrate and characterise improvements in anti-tumour activity will lead to more relevant potential clinical benefits thus directing clinical development.
In this license we will be testing the combination of potential anticancer drugs and ionising radiation with the aim of improving current anti-cancer therapies. With this license we will be able to investigate if new compounds sensitise human tumours to irradiation or if new dosing schedules are better tolerated and/or more active than the current ones. With all this information, new clinical trials can be designed that eventually may change clinical practice.

How will you look to maximise the outputs of this work?

Dissemination of knowledge at conferences via poster sessions and seminars. Publication of both successful and unsuccessful data in relevant journals. We will share pre-clinical data with principal investigators to influence clinical trial designs. We work in collaboration with many scientists and groups in which we are able to share data and learning.

Species and numbers of animals expected to be used

- Mice: 11000
- Rats: 1100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use animal studies in mice and rats alongside many other experimental approaches and they are crucial in building up a complete picture of cancer biology. Our research using animals has helped drive advances in cancer treatment that are benefiting people with cancer all over the world today.
Our work mainly uses mice (90%), which can grow tumours which mimic those of human cancer patients. Studies of cancer in mice mimic the complex way tumours grow and spread in people with cancer.
Mice can be easily genetically altered to allow us to study the genetic causes of cancer and reproduce tumour types which naturally occur in humans in the correct tissues and body systems.
We also conduct some studies in rats (10%). Some compounds that we want to test may not have sufficient levels in the blood to have an effect on the tumour in the mouse and therefore we need to use the rat as an alternative species. The rat is also usually the species of choice for toxicity studies. These studies would be conducted under a different project licence but it may be necessary to directly compare the dose level of a drug that causes an effect on the tumour to the dose level that produces unwanted side effects. This is to ensure that there is a big enough margin between activity and safety.

Typically, what will be done to an animal used in your project?

In a typical study animals are implanted with a tumour. As our work is not focused on one area of cancer but across all cancer types with focus on lung, prostate, breast, ovarian, pancreatic, bladder and haematological cancers there are a wide variety of tumour types that may be used within this licence. The majority of tumour models are derived from human tumours. As the human tumour tissue is foreign to the animal, the animals immune system would reject the tumour tissue and therefore we need to grow human tumours in either mice or rats that have an impaired immune system. This allows the tumour tissue to grow and not be rejected.
Tumours are usually implanted as cells using a needle, this is done on the lower left or right side of the back (flank) of the animal. In some cases the tumour is not available as cells and therefore a very small piece of tumour tissue needs to be surgically implanted into this area. This is done under anaesthesia and requires a very small cut in the skin and a pocket made under the skin where the tumour tissue can be placed. The cut is then sealed using either stitches, special tissue glue or clips. This area also allows the tumour to be easily monitored and measured and does not affect the animals ability to move around.
Once the tumour has started to grow the animal's tumour will be irradiated using an x-ray machine. Only the area where the tumour is will be exposed to the x-ray beam, the rest of the animal will be protected with a lead shield. The animals will be anaesthetised to ensure that the animal does not move whilst being exposed to the x-ray beam. The animals may also receive doses of an anti-cancer drug. In a typical study an animal may receive one dose of radiation each day for 5 days. Anti-cancer drugs are usually dosed orally and the animal may receive up to two doses per day for 28 days. The animals are closely monitored every day and body weights and condition of the animals are recorded to ensure that the animals are healthy. The effect on the tumour growth is compared to an animal which does not receive any active drug and/or radiation. The hope is that the anti-cancer drug in combination with radiation significantly reduces the growth of the tumour compared to either giving the drug alone or radiation alone.
During the study blood samples may be taken. This is to check the level of the drug in the blood.
At the end of the study the animal is killed and the tumour tissue and other tissues are taken which can then be

used for further investigation.

What are the expected impacts and/or adverse effects for the animals during your project?

The tumour is continually monitored and measured and although the tumour may continue to grow this does not appear to cause the animal any pain or discomfort and they continue to behave normally. The size the tumour can grow is limited by the use of a measurement/condition/size scoring system to ensure that it does not cause any pain or discomfort to the animal.
The doses of radiation are targeted to the tumour area only and the rest of the animal is protected therefore no adverse side effects are expected.
The dosing procedure for dosing of the anti-cancer drugs does not usually cause any issues but the drug itself may have some side effects. Side effects may include weight loss or abnormal behaviours such as being less active or not socialising or interacting with their cage mates. Very strict criteria are put in place to minimise any unwanted side effects to avoid any pain, suffering or distress to the animals. The side effects usually only last for a short period of time.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately 90% of animals used within the licence will be mice and 10% rats. It is expected that 90% of both the mice and rats will be returned within the moderate category and approximately 10% within the mild category. This will be for all strains of mice and rats used within this project licence.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are needed in our research to help us understand the mechanisms that underpin cancer, such as the growth and spread of tumours, and to develop new ways of diagnosing, treating and preventing the disease. Cancer is a very complex disease and animal studies are essential to understand these complexities within living organisms. They are also required by regulatory authorities before any trials of new drugs can be tested in humans. Animal studies are only performed after every feasible test has been conducted on cancer cells in the laboratory and where no alternative exists.

Which non-animal alternatives did you consider for use in this project?

Multi-cellular 'organ on a chip' models are available, but as yet have not reached the reliability and multi-system complexity of the rodent model, especially when shaping the treatment of patients in the clinic. Non-animal alternatives are used in the identification and selection of compounds. These generally include measurements of the drug's activity on particular target cells. Activity in particular cell types however cannot predict the activity

in humans due to a complexity of issues such as availability of the drug in the body and whether it is able to reach the target cancer cell.

Why were they not suitable?

They are not suitable because they cannot mimic the living organism and the processes that under-pin cancer in a living organism.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This licence reflects a well-established cancer research program and the numbers of animals used within this project licence are based on the diverse areas of cancer that are being investigated.
We typically run approximately two studies per month, each study usually has ~100 animals per study.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

All studies are designed to ensure that the minimal numbers of animals are used to achieve the question being asked. This is done with help and guidance from a statistician who is a maths expert who uses huge amounts of data to figure out how likely it is that something will happen or not. They ensure that all studies are designed to ensure that we are able to use the minimal numbers of animals to see an effect of a potential anti-cancer drug if there is an effect.
Good experimental design principles such as randomisation are incorporated into all experiments. All study designs are approved by a statistician.
All experiments are performed in accordance with Good Laboratory Standards (GLS). This standard sets the minimum laboratory requirements for all our research and development. This ensures that procedures and results are accurate, reliable, traceable and reproducible and where appropriate, comply with the appropriate regulatory authorities' legislation.
All experiments are performed in accordance with the PREPARE guidelines - Planning Research and Experimental Procedures on Animals: Recommendations for Excellence.
All research that will be published will be published in accordance with the ARRIVE guidelines - Animal Research: Reporting of In Vivo Experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

When multiple project groups want to investigate the effect of their compounds in the same tumour model wherever possible we run these within one study that share the same control group therefore reducing the requirement for multiple control groups if the studies were run independently, leading to an overall reduction in numbers. Such opportunities are identified at monthly demand meetings I chair where projects are looking at exploring their compounds in combination with radiotherapy treatment.
To minimise any side effects associated with treatment of potential drugs, a small pilot study in 2- 3 animals is

performed to ensure the treatment does not have any unwanted side effects before progressing into larger numbers of animals.
Wherever possible multiple tumour and/or tissue samples will be taken from the same animals and may be frozen down and used in other non-animal experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use a wide variety of different tumour models in both mice (90%) and rats (10%). The majority of the tumours are implanted as cells in an area on the animal (the lower left or right side of the back (flank)) which allows the tumour to be easily monitored and measured and does not affect the animals ability to move around. The size the tumour can grow is limited to ensure that they do cause any pain or discomfort to the animal.
The doses of radiation are targeted to the tumour area only and the rest of the animal is protected therefore no adverse side effects are expected.
The dosing procedure for dosing of the anti-cancer drugs does not usually cause any issues but the drug itself may have some side effects. Side effects may include weight loss or abnormal behaviours such as being less active or not socialising or interacting with their cage mates. The drugs are tested in a very small number of animals initially (2 to 3 per group) and only drugs that do not have unwanted side effects can be used in larger numbers of animals.

Why can’t you use animals that are less sentient?

Using less sentient animals for example a non-mammalian species such as the fruit fly, is not possible since they lack a closed circulatory system and so you cannot replicate a number of the complex processes that underpin cancer such as the growth and spread of cancer.
Earlier life stages of vertebrates such as zebrafish is an option, and early studies of human xenograft models do show promise of tumour inhibition studies . However the small size and difficulty of collecting meaningful blood samples makes pharmacokinetics essential in our work, currently impossible in that model.
https://www.sciencedirect.com/science/article/abs/pii/S2405803320301217 Xaio et al.

2020 Trends in Cancer (Online 17th April)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6234738/

https://www.sciencedirect.com/science/article/pii/S2352396419307881 Costa et al 2020 in the EBioMedicine (The Lancet) Developments in zebrafish avatars as radiotherapy sensitivity reporters — towards personalized medicine.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals will be acclimatised for 7 days from arrival before they undergo any experimental procedure.
Animals will not be handled by the tail and will be handled by an alternative method, for example tunnel handling, modified cupping and/or ‘pinch’ scruffing. The tail will only be used for the initial catching of an animal in exceptional circumstances or when absolutely necessary (for example if an animal has escaped and priority is to regain control).
All surgery is performed in concordance with 2017 LASA Guiding Principles for Preparing for and Undertaking

Aseptic Surgery. Any animals that undergo a surgical procedure will be provided with analgesia (pain killers) prior to the surgery and maintained in a warm environment until full recovery to minimise weight loss. Analgesia may be administered post-operatively within an edible jelly. The mice and rats will have access to a non-medicated form of the jelly prior to surgery to become accustomed to eating it.
Explore if there are options to apply any palliative treatments to minimise any adverse effects of dosing.
All parental dosing routes will be done using a single needle. For cell implants this will be done with a single needle unless documented exceptions are in place.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidelines for the welfare and use of animals in cancer research (Workman, P., Aboagye, E., Balkwill, F. et al. Br J Cancer 102, 1555–1577 (2010))
Animal research: Reporting in vivo experiments: The ARRIVE guidelines. Br J Pharmacol. 2010 Aug; 160(7): 1577–1579.
PREPARE: guidelines for planning animal research and testing (Adrian J Smith, R Eddie Clutton, Elliot Lilley et al. Laboratory Animals Volume: 52 issue: 2, page(s): 135-141
LASA Guiding Principles

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I actively participate in continuous professional development as a fellow member of the Royal Society of Biology. I receive The Biologist newsletter every month which often provides a good source of relevant 3R's initiatives. I also follow the NC3R's website to keep myself updated on relevant 3R's initiatives (https://www.nc3rs.org.uk/news/using-award-scheme-promote-3rs-innovation). We also actively discuss and implement new 3R's initiatives and run a yearly 3R's poster session competition, sharing information across different establishments.
I am an active member of AWERB and annual refinement goals are set annually by AWERB, for example alternative mouse handling.

Home Office

NON-TECHNICAL SUMMARY

156. Radiation-induced Leukaemogenesis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Radiosensitivity, leukaemia, low dose/dose rate, Haematopoietic stem cells, metabolism

Animal types	Life stages
Mice	juvenile, adult, aged, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

In this project we aim to use mouse models to obtain mechanistic and quantitative data on events occurring in bone marrow cells which lead to the development of leukaemia following exposure to Ionising Radiation (IR). This data will eventually contribute to the development and refinement of leukaemia risk projection models for IR-exposed human populations, particularly at low dose/dose rates, and to investigate factors which may modify this risk.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Human populations are exposed to Ionising Radiation (IR) from a variety of environmental and manmade sources e.g. Radon, medical imaging and radiological accidents. Studies of Japanese atomic bomb survivors and other exposed populations provide clear support for an increased leukaemia incidence following high-to-moderate IR exposure, especially Acute Myeloid Leukaemia (rAML) and risk models have been developed for these exposures. However, to is harder to model risk at lower dose exposures due to insufficient available epidemiological data. These low dose/dose rate exposures are the most relevant to the general population and have increased significantly in recent years due to increased use of medical imaging e.g. CT scans.

rAML is cancer of white blood cells (leukocytes) of the myeloid lineage originating within haematopoietic tissue (bone marrow and spleen) which produces and maintains the blood system. This process, known as haematopoiesis, is dependent on the ability of Haematopoietic Stem Cells (HSC) to divide asymmetrically to both self-renew and to produce daughter progenitor cells (Haematopoietic Stem/Progenitor Cells (HSPC)). The HSPCs continue to differentiate, eventually producing mature blood cells of different lineages.

Leukaemogenesis is the process by which HSPC are transformed into leukaemic cells by acquisition of certain specific mutations and modifications to DNA sequences which then effect gene function and expression. It is also influenced by multiple internal and external factors, such as inherited genetics and diet/metabolism. A good understanding of how radiation-induced DNA damage occurs and how it is repaired has been developed over many years. However, more research is needed to understand how the sequence of early molecular/cytogenetic events occurring in HSPC, and the multiple factors which influence this, lead to the development of rAML. Lack of knowledge in these areas inhibits development of risk estimates and new approaches for rAML prevention or detection.

Radiation-induced leukaemogenesis is a complex multi-step process that can be difficult to study in humans due to the scarcity of appropriate samples, their relative genetic and etiological complexity and the inability to study pre-leukaemic events. Therefore, mouse models of rAML are invaluable in providing a better understanding of the molecular mechanisms of tumour initiation and development.

The work proposed in this project aims to use genetically modified versions of an established mouse rAML model to investigate radiation-induced leukaemogenesis and the factors which influence this process by monitoring the progression of initiated, 'pre-cancerous' cells *in vivo* and *in vitro* as they acquire critical characteristics in a stepwise fashion after radiation exposure towards rAML progression.

The insights gained will contribute to developing and refining radiation leukaemia risk projection models, particularly in relation to low dose/dose rate exposures relevant to the general population and will also eventually contribute to the development of Radiation Protection Health and Safety guidelines. Additionally, identifying factors which influence rAML risk is of interest to the Radiation Protection field, as this may lead to interventions which can mitigate the effect of exposure to IR.

What outputs do you think you will see at the end of this project?

The scientific outputs of this project will be new and increased knowledge of the radiation-induced leukaemogensis process, such as identification and characterisation of the target cell at risk within the bone marrow, quantitative measurements of target cell radio-sensitivity and the effect of oxygen metabolism on this process, identification of new mutations which may provide alternate leukaemogenesis pathways, and also identification of potential mitigating factors such as dietary alterations and further understanding their influence on radiation-induced leukaemogenesis development. This will take the form of scientific publications in peer reviewed journals, poster and oral presentations at scientific meetings.

Who or what will benefit from these outputs, and how?

Current risk estimates of the UK population suggest 9000 cancer cases per year are attributable to IR exposure from average annual exposure to a member of the general public of 2.7 mSv, made up of natural background radiation, dental x-rays, medical imaging etc. However over- or under- estimation is possible due to the uncertainty on the true risks and occupational doses can be higher. Information obtained in the proposed project would eventually feed into the development of refined models for risk estimation in human populations, particularly following low dose radiation exposures.
Animal studies have contributed to the evidence base for providing advice and guidance to international organisations (e.g. The International Commission on Radiological Protection (ICRP),
International Atomic Energy Agency (IAEA)), government agencies (e.g. Health and Safety Executive, Nuclear Installations Inspectorate, Ministry of Defence and Environment Agency), and the nuclear industry. Radiation Protection guidelines are under constant revision and any new information added to the evidence base will be taken in to account in these revisions. These new guidelines eventually form the basis of Radiation Protection Health and Safety advice given by Government Departments.
Additionally, improved basic knowledge of radiation-induced carcinogenesis will be obtained during this project and in the longer term this work will aid the identification of early markers of radiation exposure and associated disease. Such biomarkers can be used to identify people within exposed populations (e.g. radiotherapy patients) which may have a higher risk of developing radiation-induced leukaemia, so they can be monitored for early identification of leukaemia development, improving clinical outcomes. Additionally biomarkers can be used for biodosimetry, where quantitative measurements of biomarker presence give an accurate estimate of radiation exposure to an individual in a situation where this is unknown e.g. radiological accident. This then allows remedial action to be taken dependant on dose, such as a close monitoring of individuals at higher risk of developing radiation effects including cancer. Identification of new biomarkers of radiation exposure, particularly those which can be identified with a rapid PCR-based test is a research priority within the Radiation Protection field, as currently available methods are labour-intensive and relatively slow, so are not optimal for large radiological incidents.
Identifying and investigating possible mitigating factors for radiation-induced leukaemogenesis, such as dietary alterations, calorie restriction and availability of micro-nutrients could eventually contribute to clinical interventions for radiotherapy patients to reduce the therapy-related leukaemias risk.
Additionally, understanding the mechanism by which these dietary factors mitigate rAML risk, possibly through changes to oxygen metabolism in the bone marrow target cell, could lead to the identification of potential new theraputic targets or compounds (e.g anti-oxidants, or signalling proteins involved in reactive oxygen species metabolism) in the longer term.

How will you look to maximise the outputs of this work?

All studies that yield sound results will be submitted for publication in the open scientific literature and thus add to the knowledge base available to researchers worldwide. This evidence will be consolidated through dissemination of project results and their implications to radiation protection specialists at the stakeholder meeting in the final year of funded projects, in addition to other appropriate scientific conferences and meetings e.g. European Radiation Protection Week (ERPW).
We continually seek to collaborate with other research groups across the UK and Europe, which provides significant benefits, as we are able to provide access to tissues, cells or other biological materials from our specialised mouse models for use in experimental systems which we do not have available to us. For instance, for previous collaborations we have provided mouse AML material for Transcriptomic and Proteomic Array analysis with colleagues in Europe, which lead to a joint publication, and we are currently preparing spleen and brain samples for Whole Genome Sequencing. We also provide rAML incidence/latency data and other endpoint data to researchers specialising in statistical analysis and mathematical modelling of risk.

Species and numbers of animals expected to be used

- Mice: 6300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We breed and use specialised mouse models sensitive for radiation-induced Acute Myeloid Leukaemia for our experimental studies. These models are used because they allow study of mechanistic aspects of the rAML process which is not possible in humans or in *in vitro* cell culture, such as early genetic and molecular events. The mice are young adult mice (10-12 weeks old) when irradiated at the start of the experiments, to allow enough time for rAML to develop (between 9-18 months post-irradiation).

Typically, what will be done to an animal used in your project?

Our studies generally involve irradiating our mouse models with X-rays, which involves transferring mice in to the X-irradiator in their sibling groups in cages for the duration of the irradiation (usually no more than 10 minutes). Mice are then maintained for lifespan (up to 2 years) with twice daily health checks, or until they reach their scientific end point, when they are humanely euthanised and biological samples are collected at post mortem for use in our analysis.
Some of our mice undergo blood sampling each month throughout lifespan, which involves collecting a small amount of blood from a vein in the tail with a sterile needle.
Some of the mice will be placed on altered diets, either for short periods (a few weeks) or for lifespan. These may involve reducing the calorie intake so the mice weighs 20% less than an *ad-lib* fed counterpart, periods of fasting (24 hours) alternating with periods of *ad-lib* feeding, and addition or removal of a particular nutrient (vitamins, amino acids). These mice undergo weighing and other checks several times a week to ensure that they do not lose too much weight.
Some of the mice will undergo bone marrow transplantation procedures. This involves either giving the mice a high radiation dose to remove their bone marrow, or using a mouse strain which is engineered to have defective bone marrow, then injecting specially sorted stem or progenitor cells isolated from mouse bone marrow in to the tail vein. The mice are then monitored for lifespan with tail vein bleeding to check for growth of the injected cells.

What are the expected impacts and/or adverse effects for the animals during your project?

During lifespan studies mice would be expected to develop leukaemias and other cancers associated with radiation exposure. Twice-daily checking by trained staff and use of a welfare sheet developed after extensive experience with these types of experiment mean that virtually all of these mice are humanely killed as early as possible when symptoms are identified.
In bone marrow transplantation studies, mice are vulnerable to infection and the transplant failing. Extensive measures are in place to monitor animals undergoing such procedures, and to prevent infection and provide extra nutritional and fluid support. If, despite these measures, a mouse begins to show symptoms of infection or failure of the transplant, they are removed from the study and humanely killed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority (around 80%) of experimental mice we use will experience moderate severity.
The majority of mice used for breeding will experience mild severity (around 90%), the others will experience moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It would not be not possible to achieve the objectives of the project without using animals, because currently no *in vitro* system exists that adequately recapitulates the process of leukaemogenesis *in vivo*. For instance, it is very difficult to maintain haematopoietic stem and progenitor cells (the cell of origin of radiation-induced acute myeloid leukaemia) for more than a few weeks in *in vitro* culture as the cells begin to differentiate and lose their stem cell characteristics. Also, we wish to investigate the effect of the bone marrow microenvironment on the process of leukaemogenesis which is impossible to recreate in single cell suspensions or colony assays *in vitro*. Therefore, to study radiation-induced genetic and molecular events in leukaemic target cells weeks and months post-irradiation it is necessary to irradiate *in vivo* and harvest the cells from the whole bone marrow when the timepoint is reached. However, in some circumstances cell culture assays can be employed and these are used wherever possible. Results from animal and cell culture approaches are complementary. For instance, we can use in vitro approaches to determine the effect of individual amino acid, individual vitamins or other micronutrients on leukaemic target cells, which allows us to screen possible rAML risk modifiers before applying them to in vivo diet studies.
Also, it is difficult to obtain primary human radiation-induced AML samples to study both for ethical reasons and because of the relative rarity of these tumours in the general population. Even if samples were available it can be difficult to identify the exact exposure dose an individual may have received, and these samples cannot be used for mechanistic studies to identify early pre-leukaemic events.

Which non-animal alternatives did you consider for use in this project?

There are an increasing number of methods and techniques available, provided by specialised suppliers or described in the literature, for short and long term culture of haematopoeitic stem and progenitor cells. These include cell culture plates containing 3D matrix, which mimics the microenvironment of the bone marrow stem cell niche, availability of HSPC growth medias specially created for Mouse HSPC, and isolated growth factors and other cytokines to add to murine HSPC culture media. We are continually developing and refining cell culture conditions for HSPC expansion and long term culture in our laboratory to reduce the number of mice needed to study these cell types.

Why were they not suitable?

Most of the methods described still do not produce reliable, stable long term culture of the haematopoeitic stem and progenitor cells beyond about 6 weeks. However, we are able to use them to optimise our short term culture techniques where this is appropriate, for instance, developing assays for radio-sensitivity and metabolic changes induced by radiation exposure. We have significantly increased reproducibility and reduced previous problems by customising the media/cytokine combination used, optimising the seeding cell density and using low O_2 incubators, allowing progress with characterising cellular (proliferative/clonogenic assays) and metabolic e.g. mitochondrial/glycolytic changes, after X-ray exposure *in vitro*, replacing the need to irradiate individual mice *in vivo*.
These studies generate important data and also informs experimental design for related *in vivo* studies

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Throughout the course of this project licence experimental animal numbers will be kept to the minimum required using statistical analysis techniques with advice on this being obtained from qualified and experienced statisticians available in house and with colleagues in other laboratories. These numbers will be based on previous experience and data generated in past studies, along with published studies in the literature with the same strains or techniques. This means that numbers of mice used will be the minimum required to give sufficient statistical power to each experiment.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To determine the minimal numbers of animals needed to achieve the scientific output for each experiment, we use various resources, including related experiments or techniques described in the published literature where there is a well-established experimental design available, consultations with technical experts at specialist companies to optimise *in vitro* assay methods and therefore reduce the number of *in vivo* irradiations or donor mice needed and also the guidance available on websites such as the 3Rs and ARRIVE guidelines.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We continually revise and improve protocols for harvesting and high quality preserving of tissues and extracted biological material (e.g. DNA, RNA and protein) from experimental animals, allowing increased harvest from individual animals thus increasing the data available from each experiment. Tissues, or materials extracted from tissues, will be stored for future use, both by us and for making available to collaborating laboratories for analysis techniques we do not have access to. These improvements have also allowed the use of archived historical material for cost-effective large scale screening methods, such as DNA sequencing, unavailable when the samples were first collected, therefore reducing the need for passage or repeating of AML-induction experiments to obtain more material.
We will use pilot studies for altered diet protocols which will allow calculation of statistically significant group sizes for larger studies
Both male and female mice can be used as a source of bone marrow cells and for early event studies, thus reducing the number of mice needing to be bred.
We maintain breeding colonies of specialist Genetically Altered mice which allow study of specific aspects of the rAML mechanism. These breeding colonies contain the lowest number of animals necessary for maintenance of the colony and we will be cryopreserving lines we are not currently using experimentally to avoid over breeding

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The CBA mouse model of radiation-induced leukaemogenesis is well established and has been used by laboratories worldwide for many years. It has a consistent induction rate of 20% following 3 Gy whole body irradiation, with a very low spontaneous background rate and can be reliably used in AMLinduction, dose response and early event studies. For some of our studies we will be using dietary alterations to identify possible modifiers of radiation-induced leukaemia risk.
The whole body irradiation methods we use do not require restraint whilst being carried out, minimising the stress of handling, and our experimental model mice can be group housed under standard normal conditions throughout. The optimal radiation dose for radiation-induced leukaemia induction in this model does not produce any short term adverse effects on the mice. Some of our studies require a bone marrow cell transplant to take place where the host mouse bone marrow has to be removed. For these types of studies, where possible, we will use a genetically-altered host mouse which does not require large doses of radiation to be applied to remove the bone marrow prior to transplant. When, in certain studies, a non-genetically altered host has to be used, the higher radiation dose will be given as split doses to reduce any adverse effects produced.
We use a specialist mouse tail vein injection platform when carrying out tail vein injections and when taking blood samples from our mice, which does not require head or body restraint, reducing the stress of handling and we are able to use a very small amount of blood for our experimental uses, minimising the risk of excessive blood loss on the mice. When carrying out injections we use the smallest injection volume possible with the smallest sized needle after applying local anaesthesia to minimise pain and discomfort at the injection site.
We have developed reliable checking systems to be carried out twice a day by trained staff for early signs of leukaemia symptoms in our mice to allow for humane euthanasia to be applied as early as possible, minimising any suffering. In certain experimental studies using blood sampling we are able to identify animals in a pre-leukaemic state and are able to apply an earlier endpoint before leukaemia symptoms appear.

We will develop and refine our dietary alteration methods by using pilot studies based on methods described in the literature, allowing us to identify the most appropriate checking and monitoring systems to minimise the risk of adverse effects developing and to design experiments using the minimum number of animals possible. The methods we are using do not require animals to be individually housed, minimal handling is required, and they are monitored throughout with regular weighing and body condition scoring to ensure they remain at a healthy weight.

We have developed a scientific understanding of this model system that allows us to design experiments that produce the maximum amount of data in the minimum number of animals. We also have substantial experience in the experimental techniques required e.g. FISH, QRT-PCR, FACS

increasing the chances of success of the project. The extensive amount of genetic and proteomic data available on bioinformatic websites for the mouse make this organism amongst the best model for human disease.

Why can't you use animals that are less sentient?

We are unable to use less sentient species, or mice at an immature life stage as to meet the aim to study the mechanism of radiation-induced leukaemogenesis requires development of leukaemia with tissues and data collected throughout lifespan, or at time points post-irradiation. So we have to choose a species which develops leukaemia in the same way that humans do, and use animals throughout their lifespan.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will minimise animal suffering by identifying potential adverse effects and ensuring that humane endpoints are developed and applied under these circumstances. We have developed welfare sheets and will ensure that staff involved in the day-to-day care of the animals are trained in using them. We will use under guidance from the NVS the appropriate anaesthesia when carrying out tail vein injections and blood sampling and use trained staff to handle the animals undergoing such procedures so as to minimise stress.
Mice on protocols which lead to leukaemia development are checked twice every day to identify symptoms at the earliest opportunity. Where indicated by the protocol, animals will be housed in isolators with sterile food, water and bedding, to reduce the chance of advantageous infection.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We base our leukaemia diagnosis and symptom presentation as set out in guidelines described in the Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice
(Scott C Kogan et al, Hematopathology subcommittee of the Mouse Models of Human Cancers Consortium Blood . 2002 Jul 1;100(1):238-45).

We also use the advice on the 3Rs website (www.nc3rs.org.uk/the-3rs) and also the ARRIVE guidelines (https://arriveguidelines.org/) to refine experimental techniques/protocols and experimental design

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We regularly review the scientific literature and consult with technical experts at specialist companies for new methods or techniques relevant to our research area, particularly for improvements to cell culture techniques involving haematopoeitic stem and progenitor cells, which we may be able to apply or use in our project to generate data and to inform *in vivo* experimental design, and so reduce the number of mice we need to use. Regular attendance at scientific meetings and presentations also provides information about up-to-date methods and techniques in the field which can be used to maximise the data we obtain from our research and provide opportunities to organise collaborative projects where we can share material/tissues generated in our *in vivo* studies, increasing the scientifc output from each sample set throughout the course of the project.

Home Office

NON-TECHNICAL SUMMARY

157. Red deer and the impacts of human disturbance

Project duration

5 years 0 months

Project purpose

- (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Animal behaviour, Animal movement, Disturbance, Ecology, Reproduction

Animal types	Life stages
Red deer	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to collect fine-scale data on the activity and movements of red deer, simultaneously with high quality data on aspects of the environment that could influence those. By doing so, we aim to identify the impacts of recreational land use on red deer, and to provide insights into whether those impacts could be mitigated, reducing land use conflict.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In Scotland, red deer are important to the rural economy and an economic asset for some land managers. Many estates are also attractive destinations for recreational hikers. This can lead to conflicts between these legitimate interests, and some landowners believe that disturbance from recreational hiking changes the distribution of deer across estates, reduces deer reproductive success, affects the numbers of deer available for stalking and shifts the pattern of the deer's grazing impacts. Although human disturbance of red deer has been studied before, widely varying impacts have been reported. Moreover, it is clear that deer movements need to be studied in detail along with the factors that affect them - including human movements. By conducting such a study, we aim to bring objective data to bear on a source of land-use conflict, providing information that can be used to help relieve the conflict and promote coexistence in upland areas.

What outputs do you think you will see at the end of this project?

Outputs from this project will include:

- Data on the movements of individual deer across the annual cycle, showing patterns of habitat use and whether deer movements and habitat use vary with different degrees of disturbance.
- Additional data on the movement, behaviour, and spatial distribution of red deer.
- These data will be used to generate project outputs in the form of a PhD thesis, publications in academic journals, and various public outreach media to disseminate findings to the widest range of people that will find it helpful.

GPS collars are widely used in deer studies that investigate movement and behaviour in deer, and their value for achieving these project goals is well established in the literature. Even a modest amount of data collection, owing to unexpected adverse field conditions or unusual levels of equipment failure, will substantially improve the quantitative basis for addressing existing conflicts, moving the debate away from polarised opinion, and towards measurement and mitigation. In the highly unlikely event of substantially reduced data collection (owing, for example, to extreme weather events at key times), a demonstration of the value and potential insights to be gained from such data would reassure funders and serve as both proof of principle and pilot data for a future field campaign.

Who or what will benefit from these outputs, and how?

Conflicts arise when land-user activities are at odds. The impact of recreational land users on deer, in particular, is suggested to conflict with deer stalking in the area by dispersing deer away from the area, and by reducing deer population size via impacts on deer energy expenditure, their foraging behaviour and, ultimately, their survival and recruitment. These effects have implications for management activities, in addition to economic and ecological impacts. Furthermore, there are welfare implications for wildlife under pressure from disturbance by humans.

With this in mind, the benefits of the project will be to resolve conflict by providing objective information on the

significance of disturbance of deer by hillwalkers, and to provide informed management options to mitigate the effects of disturbance. More generally, this research is critical on both local and global scales as human recreational activity encroaches ever more into wildlife habitat. In Scotland, the fine scale movement data gathered on deer in the study site will also help to inform deer management, filling a knowledge-gap around movement at this scale.
We anticipate that these outputs will be useful, as follows: 1) to inform management decisions, for example setting culling targets that consider the additional pressure of disturbance and potential changes in deer distribution, or suggesting diversions of public footfall at key times of the year away from particularly sensitive areas – such as calving time and locations; 2) to raise public awareness of the potential impact of hillwalking on deer to encourage responsible behaviour and adherence to established paths; and 3) to contribute to the wider discussion on deer management in Scotland which is commonly lacking fine-scale information on deer movement. Long term benefits will be seen in the contribution to the development of sustainable practices for recreation and deer management to benefit wildlife and conservation, landowners, and the public.
How will you look to maximise the outputs of this work?

To test for pregnancy, we will be taking blood samples from deer whilst they are under anaesthesia. We will make residual blood (not required by our tests) available to collaborators for purposes including the genotyping of individuals for immune response-relevant genes. This collaboration maximises utility from deer captures.

Our work will lead to applied outcomes and we will seek to publish those in journals relevant to the discipline and to communicate them to stakeholders more widely through articles in industry magazines and presentations to stakeholder groups. In addition, fine-scale movement data provide a resource for behavioural ecologists with potential application to a much wider range of aspects of ecology. These include understanding monitoring and the relationships between absolute abundance and indices of abundance; parameterising predator-prey models to understand predatory success rates; and understanding how forager searching strategies mediate the relationship between area use and daily travel distance. All of these are active themes of research within the group and the data collected will be useful for advancing all of them.
Species and numbers of animals expected to be used

- : 30

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The aims and objectives of this study specifically relate to the response of wild red deer to disturbance. Alternatives, such as farmed deer, are unlikely to respond in the same way as wild deer and would fail to answer our research questions. We will focus on adult females, as (1) adults are fully independent and will be able to re-join the herd following the procedure; and (2) females - especially those with young - are particularly vulnerable to disturbance (Recarte et al. 1998, https://doi.org/10.1016/s03766357(98)00037-0), whilst condition and energy availability among adult females has a determining influence on reproduction and, hence, population growth (which encapsulates one of the ultimate impacts of disturbance).
Typically, what will be done to an animal used in your project?

Animals will be anaesthetised using a dart gun, in order to reduce stress. A collar with a GPS-enabled tracking device will be fitted. We will take a small blood sample to assess whether the animal is pregnant. We will also fit an ear tag and take an ear notch so that the animal can be recognised subsequently and not inadvertently

admitted into the human food chain. The animal's recovery from anaesthesia will be assisted with a reversal agent. The whole procedure is expected to take about 30 minutes at most. We will not attach collars to more than 30 animals over the duration of the study (a maximum of 3 years). Each collar will continue to collect data over a 12 month period and will be programmed to drop off the animal at the end of that time.

What are the expected impacts and/or adverse effects for the animals during your project?

Expected impacts include pain from the intramuscular dart injection, prior to the anaesthetic taking effect, and transient discomfort from the injections and ear notching as well as, potentially from the unfamiliar collar. We do not expect any of these transient effects to last more than 72 hours.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The procedure is expected to have mild severity for 100% of animals.

What will happen to animals at the end of this project?

- Set free

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The specific focus of the research is on the red deer in its natural habitat. It would not be meaningful to substitute it with another species.

Which non-animal alternatives did you consider for use in this project?

Direct observation, pellet group (dung) surveys to indicate space use, camera trapping.

Why were they not suitable?

This project will investigate deer movement and behaviour in time and space. An equal focus on temporal variation in movements means that typical spatial distribution methods, such as pellet surveys, are insufficient. Given our interest in direct, short-term responses to mobile stimuli, as well as the spatial scale over which we are working, camera trapping also has limited value. Alternatives such as direct observations are limited by observer bias, difficulty in tracking multiple animals over large distances, potential influence of observer presence on the deer, and the amount of time required. By using GPS collars, the project limits interaction with the deer to a short capture operation, whilst maximising data quality and quantity to build a comprehensive response to our research questions.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have been guided by precedents among published studies employing the same techniques, and by our calculations of the number of animals needed to supply enough data to answer our research questions robustly.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We are focusing on a specific demographic group (females of breeding age) to minimise unwanted variation between subjects, thereby minimising required sample sizes. We are also using collars made by a reputable company and the model is well tested in the field. Prior to deployment, all collars will be rigorously tested to ensure working condition. Data obtained from the collared animals will be maximised by supplementing it with data from additional sources (including pellet group surveys, direct observations, camera trapping and vegetation surveys) to complement GPS and behavioural data from the collars. All functions of the collars will be utilised, including the built-in accelerometer that indicates activity levels and head position, and the VHF beacon for relocations in the field. The GPS data, themselves, will provide multiple data analysis options to answer the research questions. For example, GPS locations can provide information on temporal distributions, in addition to a subset of the locations being used to determine habitat selection. GPS and activity data can also be combined to look at activity in response to specific disturbance events. Taken together, these features of the collars mean that we will need to collar fewer animals to gather large quantities of data to provide a robust answer to our research questions.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Additional measures include the use of data from related studies, that allow us to run computer simulations to bolster our confidence that the number of animals used will be adequate.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Red deer have been chosen for this study because they are the only relevant species in the context of the research question, with alternatives such as domestic ungulates or farmed deer unsuitable. Red deer, and other wild deer species, are commonly and successfully used in similar research projects involving capture and fitting GPS collars.
Methods for capture have been developed with veterinary advice to ensure they are the most refined for the purpose, including anaesthesia with recommended drug doses and combinations. A number of further steps will be taken to minimise animal suffering and stress during the capture procedure and immediately thereafter, and for the duration of collar deployment (which will not exceed 12 months).

Why can’t you use animals that are less sentient?

The focus of this study is on the red deer, which is the specific organism of economic, cultural and economic

interest in this context. The research questions and context would not apply to another species.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The protocol will be conducted by vets and will only be conducted by other suitably qualified personnel if the vets are satisfied of their competency by repeat performance under supervision, as agreed with the NTCO.
Capture and collaring will take place in winter. Specifically, we will not commence darting until 4-6 weeks after the end of the rut (mating) period (to avoid disrupting mating or early pregnancy). and we will not continue darting beyond the end of March, which prevents us from darting during late-stage pregnancy. Offspring are no longer suckling or substantially dependent on their mothers during winter. Collaring in winter also reduces the risk of heat stress in anaesthetised deer, whilst doing so before the later stages of the winter period ensures that animals are not in poor condition as a result of prolonged exposure to low food availability and low temperatures.
The primary method of capture is to use a dart gun to administer the anaesthetic to an unconfined animal. Veterinary advice is that, to ensure that enough animals can be captured, it is possible that clover traps could be used to confine the deer for a short amount of time. If confinement is deemed necessary, it will be limited to 12 hours, during which time the deer will not suffer from dehydration and appropriate feed will be available. If remote darting is not practical, anaesthetic will be administered intramuscularly to an animal once safely restrained.
We will only conduct darting where there are no features in the terrain that may cause injury on induction and recovery. Failure to re-join the herd is not a documented problem but, to avoid concerns, we will work only with fully independent adults. Using an expert and competent team for darting and collaring ensures that the time away from the herd will be kept to a minimum.
During the capture, every effort will be made to minimise stress to the deer. The deer will first be approached from behind whilst watching for eye, ear and head movements that can signal consciousness. First contact with the deer will be a light touch to the hind quarters so that the response can be safely observed, and a blindfold will be put on over the deer’s eyes to minimise external stimuli. To reduce likelihood of injury on releasing the deer, all persons will move away from the deer, allowing the deer an obvious escape route, clear from obstacles, and in the direction the deer is facing.
Monitoring using the VHF tracking beacon will be conducted during the period over which the animals are collared (because GPS data cannot be used for that purpose as they will be unavailable until the collar is recovered). Collars are purpose-designed to minimise impact on the animal (weight within recommended parameters and appropriate belt-thickness) and will be worn for no more than 12 months. At 12 months the collar will drop-off via a pre-programmed device and will be retrieved using a VHF tracking beacon for recovery of data and to prevent environmental contamination. Pre-programmed devices to allow collars to drop off without the need for recapture are commonly used in studies of this nature, and will be purchased from a specialist company. Failure rates are typically low for shorter durations (not greater than 12 months). Data from mountainous regions of Central Europe reveal a 98% recovery rate for collars that have dropped off Alpine chamois and ibex. In some studies of primates, individuals have worn collars for up to 6 years without evident problems (Klegarth et al. 2019 in "GPS and GIS for primatologists: applications of spatial analysis in primate behavioral Ecology".
Cambridge University Press). The use of automated drop-off mechanisms on the collars ensures that the collars can be collected using the VHF beacon, without the need for recapture of the study animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

There is no published best practice for this type of work but two leading studies are those of the red deer on Rum (http://rumdeer.biology.ed.ac.uk; e.g., https://doi.org/10.1006/anbe.2003.2078) and the elk in Ya Ha Tinda (http://www.umt.edu/yahatinda/; e.g., https://doi.org/10.1111/oik.05304). One of the field team for the project we are proposing here has worked on both of these studies, and we have incorporated best practice from both by reference to their field protocols.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

For more general issues around the 3Rs, we will keep up-to-date via peer-reviewed journals and contacts within relevant agencies, with which we have active collaborations. For information specifically relevant to deer, we are part of a small, informal network of deer researchers in the UK and we have communicated across this network regarding project design - both for our own and others' projects.

Home Office

NON-TECHNICAL SUMMARY

158.Refining methods for rederiving and archiving genetically altered mice

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Cryopreservation, Mice, Embryos, Sperm, Superovulation

Animal types	Life stages
Mice	neonate, adult, embryo, pregnant, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

My group's main focus is on the preservation/freezing and distribution of genetically altered mice generated around the world. We want to use the most refined animal procedures possible and minimise the number of mice used in our work. This project licence has been written to enable the development/refinement of assisted reproduction techniques (ART) such as *in vitro* fertilisation, superovulation, embryo/sperm freezing and embryo transfer. Access to robust cryopreservation techniques reduces the need for live animal movements around the world, as germplasm can be successfully sent instead. In addition, by increasing the efficiency of IVF, superovulation and embryo transfer we don't need as many mice to cryopreserve or recover individual mouse strains.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Any improvements in assisted reproductive techniques we develop will be shared with other groups in the scientific community which will help refine the procedures used on mice, as well as, reduce the number of animals used around the world.

What outputs do you think you will see at the end of this project?

This project will improve the techniques used to share unique mouse models of human disease throughout the scientific community, as well as, freezing down their embryos, sperm and oocytes for future generations of scientists. We will also develop assisted reproductive techniques that will be specifically applicable to a more diverse range of strains and species of mice, such as wild-derived mice i.e. Mus spretus and WSB. These wild-derived mice offer investigators access to greater genetic diversity than the inbred laboratory mouse and may better reflects the diversity in the human population. They are particularly useful for mapping, investigating complex traits/systems and studying evolution. Unfortunately, many of the assisted reproductive techniques that have been developed in laboratory adapted mice don’t translate well to wild-derived mouse strains. For example, the success of techniques like superovulation, IVF, embryo transfer and cryopreservation is poor which makes it difficult to rederive/exchange mice between research facilities and maximise their potential. This project licence aims to address some of these issues.

Working in conjunction with a service licence we will expand and maintain a large archive of mouse strains to safeguard the lines for the future. The sharing of gold standard mouse models should increase reproducibility of studies because the founder mouse stocks we distribute to all facilities will be identical.

Through this sharing process we will enable scientific collaborations to be established around the world in the pursuit of better understanding of gene function and the causes of human disease.

Progesterone analogues will be used to investigate the benefits of delaying parturition for a short period of time. The ability to control the time of parturition will have a considerable influence on the success of hysterectomy rederivation of germ-free mice. Under these conditions it is necessary to ensure that the donor females do not litter down before the scheduled hysterectomy is performed.

Better protocols for controlling this process have the potential of leading to an overall reduction in the number of animals needed for such rederivations.

Working on a previous licences, we have developed techniques that facilitate the exchange of unfrozen embryos and sperm, as well as, the exchange of frozen sperm on dry ice. Each of these techniques offers a practical alternative to exchanging live mice. In addition, we have improved IVF and sperm freezing procedures making these techniques more efficient and robust which has reduced the number of animals used in these procedures. We intend to continue to develop these areas during the course of this project licence. In particular, we will aim to improve our overall *in vitro* fertilisation rate using frozen sperm from 55% to over 66%. In addition, we will advance our understanding of *in vitro* embryo culture systems in an attempt to remove the need to perform surgical embryo transfers following IVF. A further long term goal for this project licence is to establish an improved superovulation technique that uses a non-animal alternative to the inhibin antisera which is currently used in conjunction with hCG and PMSG to boost ovulation rates.

All significant improvements to the protocols we use will be published in the scientific literature, presented on public websites and taught on training courses available to the wider community.

Who or what will benefit from these outputs, and how?

By improving assisted reproductive techniques and techniques for sharing unique mouse models we will reduce the need to recreate mouse strains carrying the same mutation. In doing so, we will eliminate the additional animal and laboratory costs associated with re-making genetically altered mice.

Systematic improvements to assisted reproductive techniques will enable researchers around the globe to have easier access to high quality mouse lines facilitating the study of diseases and/or furthering biological and medical knowledge.

By improving the methods used to freeze down a more diverse range of mouse lines, particularly those that are no longer part of ongoing research projects, we can reduce the number of mice being bred. By doing so, the need to reimport/recreate mice that can be quickly withdrawn from the archive will be eliminated

Technical advances will be disseminated throughout the mouse community (in the literature and at scientific meetings) so that others may also benefit from the techniques e.g. through the adoption of improved embryo culture techniques, non-surgical embryo transfer procedures, the use of improved superovulation techniques for wild derived mice and/or lab mouse strains or oocyte freezing to stream line their IVF programmes. These advances will help improve the efficiency and reproducibility of animal science in across the mouse community.

How will you look to maximise the outputs of this work?

To maximise the impact of what we do, all technical improvements will be widely published in the scientific literature, on our institutional website, during our bi-annual cryopreservation training courses and at scientific meetings. What is more, any technical improvements in assisted reproductive techniques or the techniques used to exchange mouse strains will be immediately incorporated into the procedures used on my service licence.

The technical improvements made under this technology development licence will be used to improve the services we offer to the scientific community under my service licence. The improved protocols will also be disseminated widely through presentations, training courses and websites etc. The mouse strains that we freeze down for the community under my service licence, whether funded by internally or not will be presented on our institutional website and other public websites . Where this is not appropriate for reasons of commercial and academic competitiveness, strains will be held in a private archive which will be reviewed at regular intervals with depositors being encouraged to make resources public. We also promote our services as widely as possible through information leaflets, workshops, presentations and conference proceedings. Other public websites are also used to present details of the techniques we use so the other institutions can take advantage of our leading practice.

Species and numbers of animals expected to be used

- Mice: 10,750

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This is a technology development licence designed to improve the procedures used to freeze down and distribute genetically altered mice generated by the scientific community. As such it is not possible to use any other species than the mouse for this work. To preserve mouse strains for future generations of scientists it is necessary to harvest embryos and sperm from adult animals and freeze them down using well developed methods that ensure a higher proportion of embryos and sperm we obtain are able to survive the freezing process.

Typically, what will be done to an animal used in your project?

Some animals used in this project may be bred in order to establish the outcomes of the experimental work. Other mice will be injected with hormones to induced superovulation for egg (oocyte) or embryo harvesting. An additional group of other mice may undergo an operation to allow embryos to be transferred into foster mothers who will give birth.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals may be group housed or paired, mated and subject to such other non-painful procedures required for conventional breeding. Female mice may be examined for the presence of a copulation plug and culled at a specific time point in embryo development.

There are a variety of possible impacts due to the genetic modifications carried by the mice bred under this licence but the majority of animals will be from strains that do not carry any genetic modifications.

When tissue is required for DNA genetic analysis, the mildest appropriate method of sampling will be used for obtaining the tissue e.g. ear notching. Rarely due to a technical problem in analysis or the need to determine the presence or absence of additional genetic components, a second sample may be taken.

Complications due to surgery are rare although wounds may need re-closing. If this is required it will be done under general anaesthesia. The anaesthesia is brief and the mice recover quickly without undue effects on their health or weight. Pain post-surgery is controlled by administering pain relief.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The severity of the protocols used under this licence will vary between **Mild** and **Moderate**. It is expected that less than 24% (2,500) of the mice used on this licence will be recorded under a **Moderate** severity limit.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This is a technical development licence used to support the service work we do that it is specifically designed to cryopreserve the germ cells (embryos/sperm/ovaries) of genetically altered (GA) mouse strains generated by the scientific community so they are preserved for future generations or shared between laboratories without transporting live mice.

Which non-animal alternatives did you consider for use in this project?

Because this technology development licence is specifically designed to support the service work we do cryopreserving GA mouse strains generated by the scientific community there are no alternatives to the use of mice for this project.

Why were they not suitable?

Because this technology development licence is specifically designed to support the service work we do cryopreserving GA mouse strains generated by the scientific community there are no alternatives to the use of mice for this project.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This application is for a licence renewal and the numbers applied for in this licence reflect the number of mice we anticipate needing to complete the work described. These figures can only be estimates based on the expected opportunities for technical innovation and the potential for introducing new techniques developed by other groups that have yet to be published.

However, it is anticipated that up to 300 studies (averaging 15 mice/study) will be set up involving IVF techniques. The oocytes and embryos generated from these studies will be transferred (unfrozen or after a freeze/thaw cycle) into a total of 1,600 foster mothers to test the viability of the oocytes/embryos. In addition, a further 400 foster mothers will be used to test modifications to the methods used for culturing embryos for extended periods of time to make them suitable for non-surgical embryo transfers. The pups born from these embryo transfer studies will be recorded under Protocol 1 (3,000 mice).

On average, each ovary transfer study will involve the use of 4 ovary recipient females and I plan to conduct 60 studies under this project licence.

The hormone binding studies aimed at increasing superovulation rates will be based on comparisons between potential binding molecules e.g. the study would include 10 x candidate binding molecules, tested at 3 doses (with or without pregnant mares serum gonadotrophin supplementation), plus a no-treatment control. This study would be repeated 6 times. Total number of mice used 420

Where it is appropriate, statistical tools like 'Power Analysis' will be used to calculate the number of animals required to generate a statistically robust results from experimental work.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Before starting any study in this project, data will be collected from any previous relevant studies and statistical analysis used to make accurate predictions of how many animals we will need to produce a decisive scientific result. In order to keep the number of animals to a minimum, only mice required for such studies will be bred. The efficiencies of all techniques used in this project will be subjected to

regular review to ensure consistently good results, whilst striving for technical improvements which in themselves will reduce the number of animals required to provide our services.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We ensure we use the minimum number of mice compatible with the work we are doing by ensuring all reagents are vigorously quality control tested and the laboratory staff are highly trained.

We also only use the most advanced techniques compatible with the work we do. The idea behind establishing a technology development project licence is to establish a pool of skilled people with expertise in the latest techniques so that they become/remain proficient in the latest procedures and can pass on their skills to the wider community. This will help keep animal usage to a minimum at all times.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This technology development project uses the mouse as an experimental model for every aspect it covers. The mouse is used exclusively because this project has been set up to develop/introduce improvements to the technologies used under a separate service licence when freezing down and storing sperm, oocytes and embryos taken from animals that carry unique variations in their genes. The mouse is used for this type of work because it is the lowest order of mammal that can have its genes manipulated with the precision and complexity available using modern molecular biology tools like CRISPR/Cas.

The basic methods used in this project are all well establish and well understood laboratory techniques, although we will be attempting to refine them. For example, we will inject mice with similar hormones to those used in human *in vitro* fertilisation (IVF) clinics to induce superovulation. The eggs we harvest will be used for IVF sessions that have been set up to generate fertile embryos for transfers into foster females or for freezing down. The embryo transfer techniques we use are again very similar to those found in human IVF clinics. Where surgery is performed, the operation will be conducted under general anaesthesia and pain relief will be given via injectable pain killers (analgesics). Sperm will also be frozen down for use by future generation of scientists.

Why can’t you use animals that are less sentient?

This licence has been prepared specifically to develop/introduce new technologies used under a separate service licence established to freeze down and recover genetically altered mouse strains for future generations of scientists. The mouse strains used under the service licence will have been generated as part of research into the function of genes or the causes of disease. Consequently there is no alternative to using mice for this project.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

This project will aim to refine a number of well-established assisted reproduction procedures. Breeding is strictly controlled so we do not produce any more mice than is necessary for the work we want to perform.

The majority of mice used on this project will be wild type in origin i.e. not genetically modified, even so we will use known information to assess the potential welfare costs of the mice that we breed and the welfare of individual mice is followed on daily assessment sheets when it is appropriate i.e. sick animals.

Compounds are administered by injection which is considered to only cause momentary discomfort. To minimise discomfort during injections, we have a single use needle policy which means the needles will remain sharp. To minimise bruising, we also alternate the injection site when giving multiple injections.

We are hoping to develop a technique that will make the hormone preparations more efficient. This will lead to a reduction in the number of mice we need for our work.

We have developed an improved non-surgical embryo transfer technique that can entirely replace the need for abdominal surgery when handling late stage embryos. To complement this work, we are working towards improvements in the embryo culture environment to allow earlier stage embryos to be transferred using the same procedure.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure our work is conducted to the highest standards and can be reproduced by other scientists we follow, and act on the PREPARE and ARRIVE guidelines. We also have access to statisticians who can advise on the appropriate experimental design and analysis techniques. The risk of infection or delayed healing is minimised by following the LASA guidelines on aseptic procedures (LASA 2017 - Guiding Principles for Preparing for and Undertaking Aseptic Surgery).

The risk of skin necrosis due to overtight clips or non-absorbable sutures will be minimised through training and competency assessment and checking wound margins prior to recovery.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed by reviewing the literature, attending scientific meetings and talking with colleagues in the field.

In previous years we have introduced several procedural changes that have been taken up by others in the field e.g. improved sperm freezing, IVF techniques and non-surgical embryo transfer procedures. As keen supporters of the 3Rs we have successfully submitted two grant proposals to the NC3Rs.

Home Office

NON-TECHNICAL SUMMARY

159. Regulation of blood and lymphatic vessel growth

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Endothelium, Cancer, Atherosclerosis, Transcription, Angiogenesis

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Zebra fish	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to identify novel pathways that regulate different types of blood and lymphatic vessel growth and behaviour, and to determine the role these pathways play in conditions such as tumour growth and cardiovascular disease. This knowledge will be used (by our group, by our collaborators and by others in our field and beyond) to develop and repurpose therapies to manipulate vessel growth in humans with vascular-related diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

The correct formation and function of the blood and lymphatic vessel system is essential for embryonic development and postnatal tissue repair. Conversely, incorrect vascular growth is associated with a wide range of pathological conditions in humans. The growth of new blood vessels is a necessary step in the development and spread of solid tumours, while the disorganised and aberrant structure of these vessels presents a significant barrier to effective therapy; abnormal and excessive blood vessel development in the retina of the eye is a major feature in the leading blinding diseases, and the transition of endothelial cells from a quiescent to an active phenotype is a key early event in the development of atherosclerosis (clogging of the arteries). Insufficient and incorrect blood vessel growth also contributes to many disease states, including heart disease and pre-eclampsia, whilst defects in lymphatic vessels are associated with lymphedema, hypertension and tumour metastasis, and errors in endocardial (the lining of the heart) formation and function can lead to defective cardiac valves, septa and conduction systems. Consequently, a clear understanding of the genetic programmes controlling vessel growth is key to strategies for intervention and regeneration in many human disease states.

What outputs do you think you will see at the end of this project?

Major outputs:

1) Identification and characterisation of the transcription factors and upstream regulatory pathways controlling different aspects of developmental vessel growth. Time-frame = short and mid-term.

2) Generation of novel GAA (genetically altered animal) models (zebrafish and mice). These animal models can be used as endogenous reporters for each regulatory pathways, providing powerful new tools to study how vascular regulation changes during adult stages, disease and regeneration. Timeframe = short and mid-term.

3) Identification of human diseases where re-activation of developmental vascular regulatory programmes may assist in tissue repair. Time-frame = mid to long-term.

4) Provide information leading to development of new drugs to inhibit, modulate or encourage vessel growth. Time-frame = mid to long-term.

4) High-quality high impact publications. Time-frame = short and mid-term.

5) Outreach to general public. Time-frame = ongoing throughout.

Who or what will benefit from these outputs, and how?

Those in the scientific research community working on better understanding how blood and lymphatic vessels specify and differentiate will be the first to benefit, both from the increased understanding of the different regulatory pathways, and from the new animal models created. These benefits can realistically be expected to

occur from mid-way through the PPL onwards (therefore short-term and after), as the results of our studies are published, made available as pre-prints and/or presented at meetings. Our laboratory always makes all zebrafish and mouse models freely available after publication.
Our animal models are already being used by both our own group, collaborators and others to understand blood and lymphatic vessel behaviour in disease states and during tissue regeneration. These benefits will be seen first in the short-term (primarily from models started in previous PPL) and will continue beyond this current PPL. Longer-term beneficiaries (from end of PPL onwards) will be the researchers and clinicians aiming to modulate vascular growth in a range of diseases, and the patients suffering from these conditions. These benefits will range from better models to investigate vessel growth and modulation techniques in these pathologies, increased knowledge allowing repurposing of existing drugs and treatments, and the development of novel therapeutic approaches to treatments.

How will you look to maximise the outputs of this work?

Beyond the pathological models covered by this license, we are, and will continue to, actively collaborating with other specialist groups to extend the use of our mouse and zebrafish models to pathological conditions for which we lack sufficient expertise, or time, to study ourselves. Additionally, we have and will continue to freely share these models with other groups for their own research, without any conditions.

It is anticipated that the key direct beneficiaries of the work in this proposal will be members of the academic research community and healthcare professionals. Our work may also lead in the longer term to benefits to patients in terms of treatments aimed at modulating vessel growth in conditions ranging from solid tumour growth, macular degeneration, limb ischemia and heart failure. I will liaise informally and frequently with clinical collaborators to identify results and aspects of our research with relevance to the clinic.
Our university provides an ideal environment to exploit the findings of our research. Intellectual assets and outputs are managed and protected with the help of the University's Research Services Department, which provides advice and support on all issues regarding the protection of intellectual property, including contracts, patenting and material transfers.
I constantly endeavour to increase the awareness of the vascular (and associated) community of our work, and the availability and utility of our animal models. This includes writing reviews, giving presentations at conferences and seminars at a wide range of locations and topics.
Species and numbers of animals expected to be used

- Mice: 26000
- Zebra fish: 7000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use two animal species in this project, mouse and zebrafish.
Mice
We are using mice because they are the closest model to humans that is relatively easy to genetically manipulate: mice breed quickly, and it is now simple to make genetically modified mice (for example, to generate a mouse expressing a specific gene in a specific cell type, or to generate a mouse in which a specific gene has

been deleted). This work will use both wild-type (genetically normal) and genetically modified mice. Genetic modifications will include:

- Transgenic mice expressing enhancer/promoter: reporter genes. Enhancers and promoters are two types of gene regulatory elements, which are responsible for switching genes on and off (they are often considered the "on/off" switches for genes). Reporter genes are genes that are easy to detect (e.g. the LacZ reporter gene makes a protein that gives off a blue colour when in a simple solution, the GFP reporter gene makes a protein that glows green). Enhancer: reporter and promoter: reporter transgenes contain a regulatory element (on/off switch) fused to a reporter gene so that it is easy to see where and when the regulatory element switches on and off. When these transgenes are inserted into the genome of a mouse, they generate a transgenic mouse in which the expression pattern of the reporter genes tell us where and when the regulatory element is active. This can be used to find regulatory elements, to assess their activity pattern (when and where are they off and on) and to determine how each regulatory element is controlled (e.g. which proteins bind them to turn on or off).

- Transgenic mice expressing Cre, CreERT2 or similar recombination-driving genes under ubiquitous or tissue-specific regulatory elements. They express recombinase proteins (such as Cre) which allow DNA modifications (such as deleting part of a gene) to be targeted to a specific cell type (for example, modifying a gene just in endothelial cells) or to be triggered by a specific external stimulus (for example, giving mice the drug tamoxifen turns CreERT2 from inactive to active and allows us to modify a gene at a specific time-point).

- Genetically modified mice in which one or more specific genes have been mutated or deleted. These can be mice which are engineered to have loxP sites (or similar) around specific bits of one gene (this allows recombinases such as Cre to work) or mice in which entire genes have been deleted.

- Various combinations of two or three of the above (for example, a mouse expressing Cre in endothelial cells with genetically modified loxP sites inserted into a gene).

We will use all life stages with the exception of aged mice (which are not needed for our research) because we are interested in how blood and lymphatic vessels initially form and differentiate (this occurs at embryonic, fetal and neonatal stages) and also how they respond to stimuli (such as blood flow, hypoxia or disease) during adult stages, including in pregnant mice

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Zebrafish

Our research primarily uses zebrafish embryos prior to free feeding stages and therefore not regulated by the Home Office. These embryos are transparent and their blood vessels are easy to see during the early stages of development where much of the important specification and differentiation stages of vascular development occur. It is also very easy and quick to generate genetically modified zebrafish embryos, and treatments to modify different vascular pathways can often be simply added to the water the embryos swim in.
To generate the required zebrafish embryos for our research, we breed and maintain adult transgenic zebrafish expressing enhancer/promoter: reporter genes (see explanation in the mouse section above). **Typically, what will be done to an animal used in your project?**

The vast majority of mice (approximately 90%), the only procedures will be breeding and maintenance (protocols 1 and 2), in which we investigate blood and lymphatic development after death (e.g. postmortem analysis of reporter gene expression in a transgenic mouse line, post-mortem analysis of how blood vessel development is perturbed after gene deletion). The other 10% may be subject to an additional procedure. These may include administration of substances to induce gene deletion, interfere with vessel development or label the vasculature. In some of these mice, we will also induce vessel growth or vascular dysfunction in the adult. Techniques to do this include the insertion of a matrix (into which vessels will grow) or tumour (to stimulate tumour angiogenesis), the injection of a growth factor to mimic tumour angiogenesis without the tumour cells, or the induction of vascular injury by altering lipid composition, or the induction of vascular injury by surgical procedures which either ligation vessels or grafting vessels from one animal into another. We may also place mice (including

pregnant females) rapidly into a hypoxic (low oxygen) environment. This will be no lower than approximately 9.5% oxygen and will not last longer than 6 days. Analysis will again only occur after death and will include the same type of post-mortem analysis.
For all zebrafish on this licence, the only regulated procedure will be breeding and maintenance.

What are the expected impacts and/or adverse effects for the animals during your project?

Breeding and maintenance: The vast majority of mice covered by this licence (over 90%) will not be expected to experience any adverse effects because they will only be used for breeding and maintenance. A very small percentage of these mice may suffer some adverse effects as a consequence of having a compromised immune system (for example, immunocompromised mice do not have fur, and can sometimes suffer eye infections or skin infections due to this) or the consequences of gene modification (for example, if we modify a gene which plays an important role in adult vascular growth, if is possible that mice may show significant growth deficiencies). If these adverse effects occur the animals will be killed by a schedule 1 method to ensure the duration is as short as possible.
Administration of substances to alter gene expression, vascular pathways or to label or dye cells: Impacts and adverse effects from administration of substances are expected to be rare. However, potential adverse effects could include damage from administration method (for example, administering substances to mice via a gavage tube through the mouth into the oesophagus can very occasionally cause oesophageal rupture; injections into young pups can potentially damage the stomach or induce the mother to eat the pup), adverse effects due to alterations of gene expression caused by the substances given (for example, alterations of vascular pathways may cause significant growth deficiencies), or adverse effects in direct response to types of substances given (for example, a mouse may be given a drug that causes weight loss, bleeding or tremors as a side effect). Mice will be frequently observed for meeting defined humane end-points (for example, to ensure they are not losing too much weight or failing to eat and drink) and most are not expected to experience these adverse effects.
Matrix insertion and manipulation of blood vessel growth by adenovirus-VEGF: Adverse effects from matrix insertion (injecting a matrix into the mouse that blood vessels will grow into) and manipulation of blood vessel growth by adenovirus-VEGF administration (injecting adenovirus-VEGF into the mouse ear or flank region) are considered unlikely but could potentially include inflammation at the injection site. This is expected to be short-lived. If this persists then the animal would be killed by a schedule 1 method.
Tumour growth and metastasis: Adverse effects will include tumour growth, and may also include weight loss, breathing, neurological and liver and intestinal issues. To limit adverse effects to the mice, the tumours will not be permitted to grow beyond a total GMD of 1.2cm ((Length x width x height)1/3). Additionally, mice will not be permitted to suffer excessive weight loss or loss of body conditioning, respiratory distress (e.g. difficulty breathing), obvious neurological disorders, immobility, swelling of the abdomen or obvious signs of distress. Mice will be frequently observed for meeting defined humane end-points including those listed here, and if detected the animal would be killed by schedule 1 method.
Alterations of oxygen levels: Adverse effects may include mild distress when the oxygen levels are first lowered, but this should pass quickly. Additionally, some mice may lose weight at the beginning of the procedure due to reduced feeding. The mice will never be exposed to hypoxia for longer than 6 days but are not expected to show adverse effects for longer than 2-3 days. If loss of conditioning, weight loss, lower activity or mild distress are not improved after 2-3 days the animal will be killed by schedule 1 method.
Vascular damage and grafting: Adverse effects from inducing atherosclerotic lesions by feeding a higher fat diet may include oily skin resulting in skin sores, and mild obesity. The oily skin and obesity may occur for many weeks, although changes in bedding material will be made to lower risk of skin sores. Adverse effects from surgical procedures to induce vascular damage (partially tying off an artery to change the blood flow dynamics around it) and to graft vessels include the possibility that a small number of mice may suffer haemorrhage or infection from surgical wounds or death during surgery due to graft/ligation failure. If haemorrhage or wound infection are seen, the animal will immediately be killed by a schedule 1 method.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For the mice on breeding and maintenance protocols (which make up 90% of all our mouse usage), we expect the majority (approximately 95%) to experience no adverse effects at all (resulting in a subthreshold level of severity rating). Of the rest, approximately 4% are expected to experience mild adverse effects such as minor scratches and malformed teeth that to not interfere with feeding (resulting in mild levels of severity rating) and less than 1% to suffer moderate adverse effects such as fight wounds and swollen or infected genitals (resulting in moderate severity rating).
For zebrafish on breeding and maintenance protocols (all zebrafish), we expect the majority (approximately 95%) to experience no adverse effects at all (resulting in a sub-threshold level of severity rating). Of the rest, approximately 4% are expected to experience mild adverse effects such as slight growth retardation or small lumps (resulting in mild levels of severity rating) and less than 1% to suffer moderate adverse effects such as more pronounced growth abnormalities, thinness or cataracts (resulting in moderate severity rating).
For mice in which we modify vascular-related genes and/or regulatory pathways by administration of substances, we expect the majority (approximately 90%) to experience only the mild adverse effects directly associated with the substance administration (resulting in mild levels of severity rating), such as the transient pain of injection or oral dosing. We expect less than 10% of mice on this protocol will suffer moderate adverse effects (resulting in moderate levels of severity rating) such as tremors and weight loss.
We do not anticipate adverse effects directly associated with severe blood vessel development defects, as we plan our experiments so that mice (including embryonic and fetal stages) are killed using a schedule 1 method before any vascular complications would compromise the health of the mouse.
For mice in which we inject a sterile matrix (so that new blood vessels can grow into the matrix), we expect the majority (approximately 98%) to experience only the mild adverse effects associated directly with the injection of the matrix (resulting in mild levels of severity rating). Less than 2% of mice are expected to suffer moderate adverse effects (resulting in moderate levels of severity rating), such as inflammation at the wound area.
For mice in which we induce tumour growth (primary tumours and metastasis), we expect 50% of mice to experience mild adverse effects and 50% to suffer moderate severity.

For mice experiencing induction of blood vessel growth after injection of adenoviral-VEGF, we expect the majority (approximately 95%) to experience only the mild adverse effects from experiencing an injection, resulting in mild levels of severity rating. We anticipate less than 5% will suffer any moderate adverse effects such as inflammation at injection site or consequences of using immunocompromised mice (which are nude and therefore more likely to suffer skin and eye infections).
For mice experiencing lowered oxygen levels, we expect the majority to experience only the mild adverse effects associated with changes in oxygen composition such as moderate weight loss due to reduced food intake and reduced physical activity (approximately 90%). We anticipate less than 10% of mice will experience adverse effects of moderate severity, such as more severe weight loss and loss of body conditioning.
For mice experiencing vascular damage and grafting, we expect around 50% of mice to experience mild severity (mice on lipid/diet alteration steps), and around 50% to suffer moderate severity (mice undergoing grafting or ligation).

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In vitro (non-animal) techniques can be used to test if potential enhancers and promoters are functional, and to examine which cell types they are active in. However, they are very unreliable and often do not agree with animal experiments when compared side-to-side. Further, this type of experiment cannot tell us the exact pattern of gene expression driven by each potential enhancer/promoter within the complex vasculature.
Part of the problem is that endothelial cells grown in culture (e.g. grown on a plate in an incubator) cannot adequately model the complexity and heterogeneity of the vascular network. The mature vasculature contains many different types of endothelial cells, something that is not well reflected by endothelial cells in culture, which tend to be very similar regardless of where they come from. Recent single cell analysis confirms this heterogeneity and show that endothelial cells from mice show a much higher difference from each other than they do when grown on a plate in culture in the lab.

Additionally, endothelial cells behave differently in their natural environment, where they are in tubular structures, surrounded by accessory cells and matrix and receiving intrinsic and extrinsic signals.

Pathological vascular growth (when vessel growth goes wrong or responds to a pathological situation, e.g. in response to lowered oxygen levels, in a tumour environment, in response to hyperlipidaemic or changes in blood flow) can also not be fully modelled *in vitro.*

Which non-animal alternatives did you consider for use in this project?

1. **Transfection of enhancer: reporter gene constructs into cell lines:** The ability of enhancer/promoters to drive any reporter gene activity can be measure by transfecting transgenes into cells in culture and determining reporter gene activity 1-3 days later.

2. **In vitro assays of endothelial behaviour:** Assays such as cell migration and tube formation are commonly used as in vitro proxies for angiogenesis.

3. **Perturbation of gene/pathway activity in endothelial cells in culture:** It is relatively easy to perturb gene expression and regulatory pathways in endothelial cells in tissue culture, and to determine the gene activity downstream of these perturbations.

4. **Organ-on-a-chip:** These can create 3D vascular networks and generate shear stress gradients.

Why were they not suitable?

1. **Transfection of enhancer:reporter gene constructs into cell lines:** This assays is known to not give reliable results with vascular enhancers: Many known vascular enhancers active in mouse transgenic models are inactive when transfected into endothelial cells in culture, whereas some regions that do not function as enhancers in vivo can activate reporter gene expression in vitro. Further, this assay also cannot give information

about where within the vasculature the enhancer/promoter is usually active. It can however sometimes be informative when used carefully in parallel with animal studies: where an enhancer is known to be active in both in vitro transfection studies and mouse/zebrafish transgenic models, information such as the determination of crucial cis-motifs within enhancers can be obtained from in vitro studies. This is particularly useful when studying the response to stimuli such altered flow and oxygen levels.

2. **In vitro assays of endothelial behaviour:** These assays are known to be poor mimics of events in vivo. Endothelial cells behave differently in their natural environment, where they are in tubular structures, surrounded by accessory cells and receiving intrinsic and extrinsic signals. Further, these assays do not provide reliable information about the heterogeneity of the endothelium: for example, it is impossible to mimic arteriovenous differentiation in these assays, nor to model the early specification of lymphatic endothelial cells from venous endothelium.

3. **Perturbation of gene/pathway activity in endothelial cells in culture:** It is challenging to precisely detect the effects that such perturbations have on proper vessel development, as in vitro models cannot mimic the three dimensional complex tissue of the mammalian vasculature. It is therefore impossible to use this type of analysis to understand the pattern of gene expression driven by a particular regulatory element functions within the complex vasculature: to determine the requirement of a protein or signalling pathway on a specific aspect of vascular development, it is necessary to look at the fully formed vascular network. Endothelial cells in culture also do not maintain their identity very well. Regardless of original source, they tend to adopt a constant proliferative, angiogenic-like mode, unlike the primarily quiescent human vasculature. Therefore, using a variety of different endothelial cell “types” in culture is unlikely to reflect how these different types of endothelial cells behave in vivo.

4. **Organ-on-a-chip:** These suffer from many of the same issues as transfection into general cells in culture, although the inclusion of shear stress may mean they can be used in parallel to some of our animal studies.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Zebrafish

Zebrafish numbers on this licence cover the required breeding population to provide genetically modified embryos for the following assays:

- Enhancer/promoter analysis. Examples of genetically modified zebrafish used in these assays include the lines expresses the mCherry reporter gene in all endothelial cells allowing us to see and follow the development of blood vessels in live embryos. Wild-type (normal, not genetically modified) embryos from wild-type zebrafish will also be used.

- Studies in which specific genes or regulatory pathways are modified to investigate their role in vascular development and endothelial behaviour. Examples of how this can be done include injecting morpholinos (small bits of DNA that bind and prevent specific proteins being made) into fertilised zebrafish eggs; or by adding chemical inhibitors to the water the fertilised eggs are kept in.

Gene/pathway disruption and analysis is restricted to 0-5 days post fertilisation (before zebrafish are free feeding and therefore before they are regulated) but requires adult fish to generate the embryos.
In each case, the analysis will be normally be qualitative in nature (e.g. looking at patterns of reporter gene expression activity, assessing pattern of mRNA expression in differentially treated conditions) and power calculations do not directly apply.
To assess likely numbers over the duration of the PPL we have considered that:

- Genetically modified lines used to visualise the vasculature (or other related cell types) will to limited to a single line for each transgene. Where suitable lines already exist, they will be obtained from the relevant supplier.

- For each different enhancer/promoter, we will usually maintain one-two independent enhancer/promoter:reporter gene transgenic zebrafish lines, which allows us to investigate activity at different genomic insertion locations.

- Established genetically modified lines are replenished approximately every two years.

- When establishing a novel genetically modified zebrafish line, zebrafish are bred more often as the enhancer: reporter gene expression is only stable after the F2 generation.

- For each specific project aim (e.g. establishing the regulation of lymphatic differentiation) we anticipate generating on average 2-6 novel transgenic lines.

Mice

Mouse numbers on this licence cover the required breeding population to provide genetically modified embryos, pups and adult mice in order to:

- Determine the activity of enhancer/promoter: reporter genes in blood and lymphatic vessels during normal vessel development and growth

- Investigate the consequences of gene, protein or pathway disruption on the vasculature and/or on enhancer/promoter activity

- Determine the behaviour of an enhancer/promoter, gene, protein or pathway during pathological vascular growth

To assess likely numbers over the duration of the PPL we have considered that:

- Mouse lines transgenic for novel enhancer/promoter: reporter gene transgenes will generally only be generated for enhancer/promoters that have already shown the ability to drive reporter gene expression in either transgenic zebrafish embryos or transgenic mouse embryos generated through external collaborators.

- We usually maintain one- two independent lines expressing each enhancer/promoter: reporter transgene, although a larger number of F0 (first generation, used when the founder is a male) and F1 (second generation, used when the founder is a female) lines may be initially studied in order to select the most representative lines for further maintenance

- Enhancer/promoter: reporter transgenic mice lines are usually maintained in colonies of 6-18 mice and replenished (new mice bred and used to replace older mice to prevent age-related adverse effects and reduced fertility) every 4-8 months, although numbers vary when multiple time points are required for a specific project.

We also study genetically modified mice where the gene of interest is either deleted or edited (for example, lox sites inserted in such a way that the gene/part of gene can be deleted by expression of Cre recombinase). Where suitable lines exist, they will be obtained from the relevant supplier. As the technology develops, we will also consider using genome editing technologies (e.g. Cas9/CRISPR) to generate transient gene deletions and

therefore reducing animal numbers. To determine the effects of gene modifications on the vasculature, sequential breeding is used to generate mice lines containing multiple modified alleles often requiring 3-4 generations.
Analysis of enhancer/promoter: reporter gene activity in wild-type (genetically normal) mice is purely qualitative (e.g. describing the pattern of reporter gene expression) and is not subject to power calculations. Previous publications from our laboratory have required a minimal of three replicates for each stage investigated, and journals often require the result to be replicated in two independent enhancer/promoter: reporter transgenic lines, to ensure that the site of transgene integration is not an issue.
Analysis of enhancer/promoter: reporter gene activity in genetically altered mice is usually also reported qualitatively with no statistical analysis but requires a higher n number to account for variability in the genetically altered mice (e.g. for Cre-ERT2-induced gene deletion, variability in levels of gene depletion must be anticipated because it is known that the reaction that activates this Cre recombinase is not always very efficient). This variability is Cre model-specific and can be monitored using common Cre reporter lines (in which the Cre recombinase activates a reporter gene) prior to the use of complex experimental models. In practice, we have needed between n=5 and n=20 samples for each genotype and have reported all data when publishing (e.g. all outcomes are reported with either multiple representative images, or with image for each data point).
To make a quantitative analysis of the effects of gene alterations on post-natal angiogenesis (blood vessel growth after birth), we will examine vessel growth in the retina. Angiogenesis occurs naturally in the retina in the first 10 days after birth, therefore this analysis reduces the need for invasive or artificially induced angiogenic assays. Vessel development in the retina occur at well defined, stereotypical points after birth, and therefore only limited time-points will be needed. The units of measurement are vascular density (how much total retina area is covered by vessels), number of branch points (how many branches each blood vessel has), and vascular outgrowth (how far from the middle of the retina does the new vessel network extend at a particular time point) within the retinal vasculature. Although power calculations are harder to define prior to pilot experiment of extent of phenotype, animal numbers are initially estimated assuming 10% standard deviation within control group, 25% standard deviation for each mutant, a difference in mean of 30% and p value of 0.05 using 1-way ANOVA or similar, requiring approximately 6 samples/group/time point.
When studying pathological vessel growth, most analysis will again be qualitative, but some measurements (e.g. tumour size and weight, vessel density and number) will be quantitative, and require statistical analysis. It is anticipated that all measurements can be taken from the same set of experiments. While the exact numbers of animal required for statistically significant results depend on the variation within and between groups, it is anticipated to need between 4-10 mice per experimental group (comparing the normal mice, known as wild-type, with the genetically modified mice). For example, with an anticipated 50% difference between groups (SD set at 25%), we need 5 mice/group to achieve 90% power, whereas for an anticipated 20% difference (SD at 15%), we need 10 mice/group for 90% power. Where possible multiple genetically modified lines will be compared to the same set of controls.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

- The analysis of putative enhancer/promoter activity and gene/pathway modification in F0 (transient) transgenic zebrafish (before 5 days post fertilisation) is used here as a partial replacement of mammals at more sentient, licensed stages. This therefore represents a reduction of our overall animal use.

- Where possible, mice will be bred as homozygotes to minimize excess waste.

- EDA analysis has contributed to the design of the analysis in gene perturbation experiments and pathological analysis. In these cases, local statisticians have also advised on power calculations, and the relevant statistical analysis required to provide meaningful results.

What measures, apart from good experimental design, will you use to optimise the number of animals

you plan to use in your project?

- I have attended a talk on efficient breeding and put the advice into practice. In general, breeding cages are separated after 4-5 litters and retired at six litters.

- For wild-type lines and those shared with other labs/outside suppliers, we assess whether it is more efficient to buy/import animals in than to generate them in-house

- Where possible, mouse lines are frozen down. In cases where we do not anticipate usage in the next 6 months, lines are not maintained.

- We perform breeding calculations before we plan our experiments and only produce the numbers of animals that we need.

- Animal numbers are recorded on computer databases in real time and are monitored regularly.

- Some pathological vascular analyses require that enhancer/promoter: reporter genes are investigated in immunocompromised mice (e.g. Foxn1 null). These lines are maintained as enhancer/promoter transgene positive, immunocompromised null backgrounds (e.g. Foxn1 -/-; enhancer:lacZ+/WT) to reduce animal numbers when breeding (as Foxn1 -/- females cannot lactate).

- We ensure any welfare or husbandry requirements of the strain have been identified. The cage card alerts people to any special welfare or husbandry requirements.

- Tissue samples are shared, or analysed for multiple different assays, where possible. For example, wild-type enhancer: reporter samples can be used as controls for multiple different assays, whereas analysis of tumour samples in enhancer: reporter lines can provide information about enhancer activity, and blood vessel behaviour (e.g. by immunohistochemistry prior to reporter gene staining).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Choice of species and models

Transgenic mice (e.g. expressing enhancer/promoter: reporter transgenes) are normally generated on a C57/Bl6; 129/J background and bred into a C57/Bl6 background. Genetically altered models imported from elsewhere are typically obtained and maintained onto the C57/Bl6 background, also sometimes strain-specific phenotypes will result in different genetic backgrounds being used. New breeding mice are introduced each year to prevent genetic drift of C57/Bl6 mice. Immunocompetent strains may include Foxn1nu mice and will be bred as heterozygotes where possible to reduce adverse effects. Where null mice have detrimental defects, efforts will be made to use tissue-specific and/or inducible models to reduce impact. As new technologies are generated to efficiently create multiple targeted alleles into one mouse line, these will be incorporated into our research to reduce wastage in breeding (e.g. where multiple alleles would typically be bred into a single mouse,

future technologies utilizing gene editing may be able to generate a F1 mouse with all desired alleles).

Matrix and tumour models to investigate angiogenesis

Aseptic precautions will be taken to reduce the risk of infection and care will be taken to ensure the animals are properly restrained during injections or incision. Injections will be used where practical to minimise distress of general anaesthesia. The matrix angiogenesis assay permits modelling of vascular growth in a healthy mouse after a minimally invasive procedure. Other models, such as wound healing and hindlimb ischemia, would cause a greater level of suffering in the mice. This model also allows for quantitative analysis of the resultant vessel growth. Occasionally slow release pellets containing tamoxifen or placebo will be used in conjunction with matrix or tumour models, or substances will be included before implantation, or injected after implantation, into the matrix (e.g. siRNA, growth factors), enabling us to alter gene expression specifically at the site of implantation. Administration of substances, including inhibitors (e.g. VEGF pathway antibodies) will permit the investigation of the role of these substances in these clear angiogenesis models.

Use of Ad-VEGF tumour surrogate models

The Ad-VEGF tumour surrogate model represents a refinement, as it simulates the tumour environment for blood vessel growth without subjecting the mouse to any tumour burden. For now, tumour passage will also be used for comparison, but as this model is developed in our lab, we anticipate moving to a position where most of our tumour angiogenesis modelling will utilize this technique with anticipated welfare benefits for the mice. The ear and flank will be used in order to match both published and unpublished data using this technique, reducing the total number of mice used as some experiments will not need to be repeated (e.g. the effects of VEGF inhibition, and time-points affected, have already been reported for Ad-VEGF injection in ear and flank, and we have access to extensive microarray data from flank regions after Ad-VEGF injection).

Models of vascular grafting and ligation

Models of vascular grafting and ligation provide valuable mechanistic data on the pathologies associated with these conditions that is hard to obtain from clinical samples due to the scarcity of this tissue and the lack of appropriate controls. The combination with our enhancer/promoter: reporter gene transgenic mice allows us to investigate the behaviour of different regulatory pathways in these models without further need for additional interventions. The pathology associated with these surgical models can be exacerbated by poor surgical technique and the length of surgery resulting in increased variability and decreased statistical power. We have worked hard to refine the surgical technique to increase consistence and decrease variability. Whenever possible we now carry out this graft surgery with two operators one to harvest the graft and one to construct the graft. This decreases total surgery time and graft ischemia resulting in reducing variability. Cohorts are designed to enable where possible all mice within one cage to undergo surgery on one day enabling mice can be maintained in a grouped housing environment.

Why can’t you use animals that are less sentient?

Much of our early validation and analysis of enhancer/promoters often occurs in transgenic zebrafish. This analysis is done before zebrafish are free feeding and are therefore not covered under ASPA. Live imaging allows study of the formation of the vessel system in the same fish, up to three different enhancer/promoter/reporter gene constructs can be examined in each transgenic embryo, and can easily be treated with chemical inhibitors, or used in morpholino or genome editing-based gene knockdown studies, enabling quick validation of putative regulatory pathways. However, studies of pathological vasculature often require a mammalian model: tumour growth and vascularization in zebrafish embryos is not a particularly good model for human disease, whilst human-relevant hypoxia is also challenging to model in zebrafish. Further, zebrafish are not an accepted model for atherosclerosis, and flow regulation in fish is widely agreed to utilize different pathways to that of mammalian flow regulation.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

- Animals will not be kept beyond 12 months of age to avoid unnecessary adverse effects associated with ageing.

- We currently use injectable anaesthetic in place of inhalation anaesthetic when injecting Ad-VEGF into mouse ears (Protocol 8) as our previous experience found it was impossible to give the inhalation anaesthetic and access the ears correctly for the intradermal injection. However, we will now trial using small moulded silicone inhalatory face masks, which would allow us to take advantage of the benefits of using inhalatory GA over injectable for this short procedure.

- Where practical (e.g. in studies of blood flow-related gene regulation) we will first screen putative enhancer/promoters in an in vitro system (e.g. parallel plate flow chamber) before producing new genetically modified lines.

- We will provide cage enrichment (e.g. sizzle nest and polycarbonate tunnels as standard, chew toys for single housed stud males, smart houses and nestlets for breeding pairs and pregnant females) to promote natural behaviours and improve general welfare.

- When possible, we will add companion female(s) to singly caged pregnant mice.

- Cryopreservation of new lines will occur early in the breeding programme.

- Bedding materials will be altered when needed, for example using Alpha Dry bedding to reduce/prevent the development of skin sores and reduce eye issues in nude mice and those feed with high fat diet.

- Mice at risk of tooth overgrowth due to high fat diet will be supplied with wooden chew blocks.

- We will trial the use of skin swabbing (for mucus) in place of fin biopsy when genotyping zebrafish.

- We will read and follow relevant LASA publications and guidelines, including those on undertaking aseptic surgery and transgenic models

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow advances publicised in the NC3R newsletter. This provides information on the most refined techniques, including new guidelines on non-aversive methods of picking up mice, single-use of needles and blood sampling. We will adhere to updated ARRIVE guidelines on reporting work with animals as now required by many journals. We will also follow publications from LASA on areas relevant to this work (e.g. guiding principles on aseptic techniques, position paper on transgenics).
Where new experiments are being set up, we will also use the online NC3Rs Experimental Design Assistant.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

- I, and all personal licence holders attached to this PPL, will be expected to attend all of our local Gold Standard meetings

- I subscribe to the NC3Rs newsletter, and discuss relevant points in our weekly lab meetings
- All PILs attached to this licence will be encouraged to attend symposia on the 3Rs (offered yearly by our University), which I will also attend.
- Periodic reviews of the literature will be held in weekly lab meetings to ensure recent advances are not overlooked

Home Office

160. Regulation of cell polarity in vertebrate development

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Xenopus laevis	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project is to use frog embryo cells to understand how tissues and body structures are built with a view to applying this basic knowledge to create realistic test tissues for medical/pharmaceutical

research or to guide biologically informed techniques for repairing diseased or damaged tissues and structures in patients.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Repair or replacement of diseased or damaged tissues and organs created from cells in the laboratory is an achievable but currently distant objective for most tissues. One of the major challenges is to reconstruct the normal architecture of tissues. The basic biology of how normal tissue architecture is generated by cells during embryonic development is the major area of investigation of this project. In particular, how do tissue achieve organised shapes by directional growth? How do the cellular components we know of that control cell polarity organise polarised cells in intact tissues? How do these relate to the action of chemical signals that influence embryonic development in general?

What outputs do you think you will see at the end of this project?

We foresee two main outputs from this project. One is a paper or several publications on the ways that self-organisation can be cued or triggered to achieve specific tissue shapes. A second is a rigorous evaluation and potentially new guidelines for the community on the optimum use nd re-use of our animal species, Xenopus leavis, potentially reducing numbers in use by up to 50%.

Who or what will benefit from these outputs, and how?

In the short-to-medium term, the research will push the field forwards, providing a better understanding of the basic biology and potential practical application of cell self-organisation to make anatomical and tissue structures. In the long term, this project will contribute to the goal of extending our ability to general specific cell types in the lab to generating or repairing structures in the lab or in patients.

How will you look to maximise the outputs of this work?

All findings will be expeditiously published in appropriate peer-reviewed journals with the maximum visibility and impact. In addition, in collaboration with NC3Rs and the XenBase community pages and at domestic and international scientific meetings, improved protocols for 3Rs will be promulgated throughout the international community of Xenopus researchers.

Species and numbers of animals expected to be used

- Xenopus laevis: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Xenopus laevis frogs are a widely used experimental animal because they generate large numbers of eggs (frog spawn) that develop into tadpoles quickly (in about three days). The frog embryos are useful for scientific study because they are visible and accessible while they develop (unlike, for example, mice or other mammalian species) and yet their development has much in common with that of other vertebrates such as humans. Understanding embryonic development is the foundation of using cells, such as stem cells, for the repair and regeneration of diseased, injured or aging tissues.

Typically, what will be done to an animal used in your project?

Female Xenopus frogs will get a hormone injecting that triggers them to lay eggs. They will then be rested for three months before the procedure is repeated. Frogs that are used and re-used this way remain vigorous and healthy in many labs around the world for many years. Some frogs will be reinjected 7-10 days after an initial injection to induce a second round of egg laying. This is similar to the interval between mating events in the rainy season in the wild. We have been piloting this approach in the lab for the past few years and the frogs appear to be just as healthy after multiple cycles of this double-egg-laying procedure as the standard procedure while generating twice as many eggs per animal. Animals will be closely monitored for any stress or cumulative effects of this double-egg-laying procedure.

What are the expected impacts and/or adverse effects for the animals during your project?

No adverse impacts or effects on the animals subjected to these procedures are expected. Historically, animals subjected to repeated superovulation re-use remain healthy and continue to grow (slowly) over many years in our colony and similar Xenopus colonies around the world.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All of the animals will be subjected to the Mild level of protocol severity.

What will happen to animals at the end of this project?

- Kept alive
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Trying to understand how cells in living tissues function and behave absolutely requires studying living tissues. These can only be obtained for this type of study from live animals.

Which non-animal alternatives did you consider for use in this project?

This project builds directly on a large body of knowledge and an experimental protocol that only exists for embryos of this animal species. Recently, some mammalian cell culture methods have begun to be used for related studies

Why were they not suitable?

The cell culture systems than are closest potential alternatives to the proposed project are too heterogenous in both composition and cell behaviour to provide the clear cut answers that this project will provide. They are also explicitly not an alternative for the development of the new ovulation procedure that this project aims to test.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have used pilot data from collaborators at the University of Chester to estimate how big the differences might be between different procedures we aim to test, and also how variable they will be. This enabled us to calculate how many tests, and therefore how many animals we need to use, to make sure that we can draw firm conclusions from the experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The re-use of animals is a major factor in reducing numbers being used and this is a central feature of this project and each of the protocols. We also used the calculations described above to determine the minimum number of animals we need to use to ensure that the scientific endpoints will be achieved with sufficient statistical power so that animals will not be wasted.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Our main measures for optimising the numbers of animals to be used are the calculations described above plus the re-use of animals (which we know to be benign) so that none have to be killed.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Xenopus leavis females can produce large numbers of eggs throughout their long lives. They are well adapted to hormone-induced ovulation (our protocol) and are used routinely this way with no evident ill effects in research institutions throughout the world.

Why can’t you use animals that are less sentient?

The research is aimed at identifying methods and rules for the self-organisation of tissues relevant to humans. Xenopus laevis is the least sentient vertebrate species useable for this work.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Stress monitoring and improved methods are an explicit component of this project. Our routine animal care and veterinary supervision (involving NVS sign-off for every re-use as well as routine monitoring) are already of the highest standard.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow both NC3Rs and ARRIVE guidelines in experimental design and our protocols have been stringently peer-reviewed according to these criteria.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We are leaders in proposing and being funded (by NC3Rs) for 3Rs work and are in touch with the NC3Rs on a regular basis.

Home Office

NON-TECHNICAL SUMMARY

161. Regulation of female fertility in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Ovarian function, Fertility preservation, Primary ovarian insufficiency, Cancer, Conservation

Animal types	Life stages
Mice	adult, pregnant, juvenile, neonate, aged
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to investigate how ovaries function by analysis of the interactions between the developing eggs and the cells of the follicle to develop treatments for human ovarian dysfunction and for conservation of endangered species.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Normal ovarian function and the generation of functional eggs underpins female fertility and the work in this project has numerous target groups. The number of children surviving cancer treatment is increasing but so too is the number of children who have undergone gonadotoxic chemotherapy or have had ovarian tissue frozen for fertility preservation. For these children, understanding how the ovarian tissue responds to different chemotherapy regimens and investigating molecules that may prevent damage is crucial. For patients with blood cancers that cannot have the tissue transplanted back, we need to develop methods to culture this tissue to grow eggs in vitro. For the 1% of women under 40 years of age with premature ovarian insufficiency (POI), 70% of the causes are unknown. We need to understand the mechanisms that cause the onset of POI and develop techniques to develop treatments. Finally, understanding how follicles develop and ovarian function is regulated will help our work into developing eggs in culture for endangered species.

What outputs do you think you will see at the end of this project?

At the end of this project, our aim is to have generated new publications revealing a deeper understanding of ovarian function in health and disease and progressed towards the development of new techniques for the clinical treatment of ovarian dysfunction including primary ovarian insufficiency (POI) and fertility preservation for ovarian cancer patients.

More specifically:

1 – Furthering our understanding about how follicle development and ovarian function are regulated throughout life, will further our understanding of ovarian function. Understanding the mechanisms that underlie follicle development, ovarian function and fertility regulation may reveal therapeutic targets for fertility treatments for women and for other species including endangered and agricultural species.

2 – By analysis of ovarian function in health and disease we will further our understanding of mechanisms that are involved in ovarian dysfunction and potentially identify mechanisms which will lead to new treatments.

3 - By developing novel methods of fertility preservation this will improve health and wellbeing for women who have fertility problems or have treatment that causes fertility problems. Since infertility causes emotional stress and can lead to mental health conditions as well as cardiovascular and skeletal issues, this will lead to improved health and wellbeing for women and girls with fertility issues.

4 – By investigating fertility preservation methods for cancer patients and how chemotherapies affect ovarian function and potential fertoprotect substances, we hope to generate data that will ultimately lead to improved methods of fertility preservation and new non-invasive treatments to preserve fertility for patients during cancer treatment.

5 – In exploring follicle development for non-domestic species including endangered species where little information exists, we will be furthering our understanding of how eggs are generated in endangered species which will support development of assisted reproductive technologies to generate eggs for endangered species.

Who or what will benefit from these outputs, and how?

These studies revealing more about ovarian function and disease will benefit scientists working in the fields of human, domestic animal and endangered species ovarian physiology whilst the work is ongoing as the data will be shared at conference presentation and by publication. The benefit to researchers is considerable and immediate upon sharing of results considering the fundamental mechanisms of ovarian function will be explored and thus will contribute to many areas of research.
In the long term, the output from this work will help women and girls with ovarian dysfunction, undergoing chemotherapy treatment, and undergoing fertility preservation for other conditions of ovarian dysfunction. The number of women and girls who survive cancer who would benefit would be considerable. The studies may lead to clinical treatments that preserve fertility for all prepubertal girls and reproductive age women who have to undergo chemotherapy. To restore or maintain fertility in women will lead to considerable economic benefits regarding their future medical requirements. The improvement to women's health by preventing early menopause caused by these diseases and cancer treatments will lead to savings for the NHS since hormone replacement therapy (HRT) treatment, treatment for osteoporosis, cardiac and metabolic conditions is reduced. Preserving fertility for female cancer survivors has huge potential for economic impact due to reduced costs for ovarian cryopreservation, future assisted reproductive techniques and IVF and treatments for side-effects that occur due to premature menopause.
The improvement and development of fertility preservation techniques to help patients with fertility will be realised after publication and the progress to developments and benefits will be pursued but will take some years for full development to clinical treatment.
Output from these studies into non-domestic species will benefit the survival of endangered species including rhino that have been pushed to the brink of extinction by poachers and thus require human intervention. Saving endangered species would lead to economic benefit for numerous counties where these species exist, there are also immeasurable conservation benefits to humankind and the planet as a whole.

How will you look to maximise the outputs of this work?

We will aim to maximise these outputs via the usual academic means including publication of results and dissemination at conferences. We will also publicise in the mainstream media and use social media to ensure the general public are aware of the advances generated by this work.
Where required to facilitate the translation of the techniques to clinical treatments, collaborations will be established.

Species and numbers of animals expected to be used

- Mice: 15,000
- Rats: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The regulation of ovarian follicle growth and development is complex and requires organization on multiple levels. To unravel the function of the oocyte requires the use of whole animals because the oocyte and follicle are mutually dependent and cell lines do not exist. Cell culture assays will be used wherever possible to characterise the function of molecules involved in oocyte-granulosa communication.
To investigate ovarian dysfunction and disease, models of such conditions are required, for example, to investigate primary ovarian insufficiency (POI), a mouse model of POI is used, and to develop fertility preservation therapies for ovarian cancer, a mouse model of ovarian cancer is used.
To examine follicle development in ovarian tissues, tissues will be transplanted into immuncompromised host animals; these animals have a dysfunctional immune system and this prevents tissue rejection after transplant.
Mice with labelled cells are used to trace cell lineages.

Typically, what will be done to an animal used in your project?

The vast majority of animals are born, maintained and humanely killed for tissue collection. To investigate follicles as they develop in the ovaries, imaging under general anaesthesia will be carried out on multiple occasions. A selection will be used as hosts for transplanted ovarian tissue from other species including human, sheep, rhino and mouse. Human ovarian tissue will not be transplanted in the ovarian bursa but into other locations for study; most often under the skin but occasionally into the omentum in the abdomen or under the kidney capsule. Transplanting up to 4 small pieces in the same animal via a limited number of surgical sites means we can carry out more meaningful experiments using less mice. Transplanting multiple pieces minimally extends surgery but does not result in any adverse effects. Some animals will undergo two surgeries far enough apart to ensure animals have fully recovered between. If follicles develop well, animals with transplants may also undergo multiple imaging sessions under general anaesthesia. The effect of drugs on ovarian function will be investigated. These will include chemotherapy drugs as well as drugs that can protect fertility during chemotherapy treatment (fertoprotect drugs).

What are the expected impacts and/or adverse effects for the animals during your project?

To study the effects of chemotherapy on ovarian function and to investigate molecules that may protect fertility during chemotherapy, we have to administer mice with chemotherapy. This will result in typical symptoms associated with chemotherapy treatments such as weight loss and ill health over the following 1-2 weeks.
To observe follicle development as it occurs normally, we will image the ovary using imaging technology such as MRI. To ensure the animals remain still and not stressed by the procedure, this will be done under general anaesthesia. To watch follicles as they develop, multiple anaesthesia's will be carried out which may accumulatively result in slower recovery from the anaesthesia.
To further our understanding of ovarian function in health and disease, we need to analyse ovaries and thus some experiments require surgery to transplant and modify ovaries under general anaesthesia. This can cause pain although this is alleviated through the use of pain killers. Typically animals recover well and behaving normally within an hour of surgery.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice:

Sub-threshold: 90% of all mice used.
Mild: 5% of all mice used.
Moderate - 5% of all mice used.
Rats:

Moderate: 100% as all animals used will have undergone surgery.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The regulation of ovarian follicle growth and development is complex and requires organisation of many different cells types on multiple levels. To unravel the function of the oocyte and the somatic cells that support its development requires the use of intact ovaries in whole animals because the oocyte and follicle are mutually dependent and cell lines do not exist.

Which non-animal alternatives did you consider for use in this project?

Cell culture assays and tissue culture will be used wherever possible to characterise the function of molecules involved in oocyte-granulosa communication. However, at present culture techniques do not fully replicate follicle development in vivo.

Why were they not suitable?

Although culture of ovarian tissues is being investigated, to date, culture of these tissue culture does not replicate oocyte and follicle development as it occurs in vivo.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers stated are based on my previous two Project Licenses.
The number of animals used for experiments will be around half of all animals bred since our work is the study of ovaries, and the only use we have for male mice (from lines with modification in ovarian function) is as breeders, and therefore, the majority of male mice are culled before weaning. Wildtype and immunocompromised males will be shared where possible.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Since the males have genetic modifications, there is no desire for them as they are not wildtype. If we breed immunocompromised SCID mice, the males will be made available to other groups. Animals required for each experiment will be carefully calculated and minimum numbers will be used to obtain statistical significance.
Bovine, ovine and equine ovaries are also used where possible since numerous ovaries can be obtained with no cost to animals from local slaughterhouses.
Ovaries collected are serially sectioned and thus many samples are available for analysis from the same animals.
We aim to reduce variation in the data by careful control of age and weight between groups. When possible the researcher is working blind to reduce measurement error.
Pilot studies will be performed when feasible followed by power tests to ascertain the number required to test for significant effects before larger experiments are carried out.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

All animals used will be used as efficiently as possible by maximising the data collected per individual to ensure that the use of each animal is maximised. Transgenic mice will be maintained as individual colonies unless they are readily available commercially and it is more efficient to purchase them. Using breeding calculations, each mouse line maintained will produce only the mice needed for the specific designated experiments and for maintaining the colony minimising the number of animals used.

Recent amendments to our previous license have enabled us to graft multiple tissue pieces into one animal during one same surgery; this results in the use of fewer mice.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The majority of our work uses ovarian tissue collected from dead animals and therefore, there is no lasting pain or harm.
The mouse models used are models of ovarian dysfunction and therefore have no adverse phenotype other than dysfunctional ovaries. Where models of ovarian cancer are used, the cancer is also targeted to the ovary, and since we are aiming to develop treatments for patients that have recovered from ovarian cancer, we will be using these models before the disease extends beyond the ovary.
To investigate the effect of chemotherapy drugs and fertoprotect drugs, these will be administered to mice. The dose and regimen will be used based on published studies with clinical relevance to human drug regimens. Mice will be carefully monitored and any side effects addressed where possible, e.g., mice with hair loss will be provided with additional bedding. Soft palatable food will be provided to all animals given drugs with some toxological side effects, as well as control animals.
To investigate the effect on human tissues, human ovarian tissues from patients that have consented to

research in animals will be used to examine the effect of these drugs and fertoprotect drugs on ovarian function.
To investigate follicle development in non-domestic species, tissues need to be transplanted into mice so that we can ascertain what 'is' normal development so that can aim to replicate it in cultured tissues.
Immunocompromised mice and rats will be used as the lack of an immune system is required to prevent graft rejection.
Transplantation of up to 4 pieces of ovarian tissues into a single animal in a single surgery with a max of two incisions means that less animals are used to obtain the same data.

Why can’t you use animals that are less sentient?

The regulation of ovarian follicle growth and development is complex and requires organization on multiple levels. To unravel the function of the oocyte in mammalian development requires the use of whole animals because the oocyte and follicle are mutually dependent and cell lines do not exist.
Mice have been chosen for these studies because they are the species of lowest neurophysiological sensitivity that will allow us to investigate these scientific questions. It is also relatively straightforward to manipulate genes in mice.
Since ovarian development changes with species of increasing complexity, to investigate mammalian development with the aim of developing treatments for patients, using fish or other less sentient models such as drosophila are not appropriate.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Substance administration will be via the least invasive technique.
Chemotherapy regimens are based on published studies and pilot studies will be carried out where appropriate.
Numerous sites for transplantation of ovarian tissue have been included so that the most appropriate can be used for each study; for example, human, cow and sheep follicles can grow much larger than mouse and therefore transplanting human, cow or sheep ovarian tissue beneath the kidney capsule to develop mature follicles is not the most appropriate.
Human ovarian tissue will not be transplanted beneath the ovarian bursa.
Numerous refinements to surgery are carried out. Animals are regularly handled before surgery to ensure they are used to human manipulation and contact so that immediately prior and during post surgery monitoring, this aspect is stress-free. The dose of analgesia for managing pain is administered based on an individual animal's weight. To aid with post-operative recovery, more enrichments in the cage are provided.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The guidelines suggested by NC3R.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We participate in initiatives of the NC3R e.g. 3R research day, we are in contact with the 3R information officer of our institution and have subscribed to email alerts. We also have regular consultations with the NACWO and veterinarians

Home Office

NON-TECHNICAL SUMMARY

162. Regulation of thrombus formation

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of the project is to investigate the cellular and molecular mechanisms involved in thrombus (blood clot) formation and ultimately to specifically block or modulate these mechanisms.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The formation of blood clots, although essential for normal haemostasis, can pose a significant risk to health. Thrombus formation is a contributory factor in heart attacks, stroke and atrial fibrillation. It is therefore extremely important that the underlying mechanisms of clot formation are better understood so that more effective therapies, and ultimately preventative therapies, can be designed. However, one of the main problems with agents used to prevent clotting is the increased risk of uncontrollable bleeding. This adds to the importance of understanding the intricacies of clot formation and lysis.

What outputs do you think you will see at the end of this project?

Through this Project Licence we will gain a greater understanding of the initiation, formation, structure and stability of thrombi and ways in which thrombus formation can be inhibited. The detail of mechanisms and molecules involved in specific mechanisms of thrombus formation and lysis can be used to develop small molecule inhibitors of these target molecules. The clinical benefits may be identification of novel pathways that can be targeted with therapeutics minimising risks associated (e.g. bleeding) with current therapies; this could be through the development of new treatments for thrombosis that are directed at modulating the developing fibrin clot rather than preventing its formation, as an alternative or complement to existing anticoagulant, antiplatelet and thrombolytic treatments. **Who or what will benefit from these outputs, and how?**

It is anticipated that the information gained through this programme of work will be of benefit to the scientific community through peer review publication. Findings gained from the in vivo work detailed in this project, together with in vitro data obtained will provide the research community with insight into the role of specific molecules in thrombus formation. They will also provide a greater understanding of alterations in function in clinically relevant mutations of these molecules.
Small molecules showing the greatest inhibition of thrombus formation in vivo will be sent for further testing with the aim of reaching human clinical trials.

How will you look to maximise the outputs of this work?

Our studies are conducted alongside other collaborators which has successfully established channels to share new knowledge to further develop and refine in vivo and in vitro systems to study thrombus formation at the cellular level. We have developed in vitro assays to examine potential test compounds on the basis of selectivity and efficacy.
Our research findings will be shared with the greater scientific community in the form of national and international conferences and workshops and also through peer-reviewed publication. **Species and numbers of animals expected to be used**

- Mice: 1900

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult mice will be used as models of thrombus formation are well established in this species. Also, selective blocking antibodies and genetically modified or mutant mice relevant to these studies are available. We also know that human clotting factors and inhibitors have physiologically relevant actions in mice. In addition to this we intend to use genetically altered mice with specific clotting factor mutations to study their effects on clot development.

Typically, what will be done to an animal used in your project?

Under non-recovery anaesthesia, the carotid artery will be cannulated.
Test compounds and Fluorescent labels administered via the carotid artery.
Femoral vein or cremaster vessels will be exposed for microscopic visualisation.
Thrombosis will be initiated by causing injury to the surface of blood vessel(s) chemically or by laser injury.
Microscopic Images of the developing clot taken at regular intervals for up to 1 hour post injury.
At the end of imaging, whole blood will be taken for plasma samples.
Mouse killed.
A small proportion of mice will receive the test compound under investigation orally, intravenously or injected under the skin prior to being used under non-recovery anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

Most of the animals used will undergo non-recovery anaesthesia to minimise suffering. If recovery is necessary, all procedures will be carried out by competent personal licence holders using appropriate procedures and experiments will be terminated should any sign of suffering be observed.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

90 % of mice will be used under non-recovery anaesthesia.
10% of mice undergo a surgical procedure under general anaesthesia and allowed to recover for a period of up to 7 days before being used under non-recovery experiments. This is to study the development of clots and the test compounds over longer periods of time. During the recovery period, the health and welfare of mice will be regularly monitored by experienced personnel.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Although initial experiments will be carried out using ex vivo models of clot formation, the use of animals is required due to the complex nature of the clotting cascade; this requires multiple factors to be present simultaneously to evaluate the complete effect.

Which non-animal alternatives did you consider for use in this project?

Platelet aggregation assays and clot lysis assays are considered and are used as in vitro studies alongside this project.

Why were they not suitable?

In vitro studies, as mentioned, provide valuable information regarding the cellular components involved in the clotting cascade but do not replicate the interactions of these cellular components within a clinically relevant biological system.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We calculate that 6 animals will be required for each experiment. These calculations will be constantly reviewed after initial experiments are performed to ensure they are accurate. If required, additional advice on study design and subsequent data analysis will be sought from our statistics service for more complex studies.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Internal controls will be used whenever possible. For example, in time course experiments progression of thrombus formation will be compared to fluorescence within the vessel recorded prior to injury. Where internal controls are not an option, test animals will be compared with inbred age-, weight- and sexmatched controls to minimize variation.
The online Experimental Design Assistant provided by NC3Rs will be used where applicable and advice from our statisticians will be sought to ensure correct number of animals are used to achieve statistical significance per study.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In vitro studies such as clot lysis assays and compound profiling studies (to analyse target specificity, potency and toxicity etc) are carried out to identify the best candidates for in vivo use. This reduced the number of compounds being put forward for in vivo analysis.
Small pilot studies are carried out for each family of compounds. Superior compounds are carried forward for

dose response studies. This ensures inferior compounds are not part of large scale investigations therefore reducing the number of animals used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice will be used as models of thrombus formation as they are well established in this species. Also, selective blocking antibodies and genetically modified or mutant mice relevant to these studies are available. We also know that human clotting factors and inhibitors have physiologically relevant actions in mice.
Non-recovery anaesthesia is used throughout our studies to reduce pain and suffering to the animals.

Why can't you use animals that are less sentient?

Cellular components of blood and Clotting factors involved in the coagulation pathways in humans and mice are closely related. We have a well-established murine thrombosis model which utilises mutant mice as well as specific clotting cascade inhibitors which are not available or compatible in less sentient species. Scientific outcomes using mice in these studies will be of greater relevance with the aim of our findings leading to human clinical trials in the future.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Most experiments carried out under non-recovery anaesthesia therefore most refined.
Animals used in recovery experiments will receive the appropriate post-operative care (pain relief administered, kept incubated and softened food provided in necessary) to reduce pain and suffering and regular monitoring until fully recovered. Any animals failing to recover with 2-3 hours post op will be killed.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The principles in the LASA guidelines will be followed http://www.lasa.co.uk/wp-

content/uploads/2017/04/Aseptic-surgery-final.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

NC3Rs workshops/symposia are held regularly and provide an opportunity to discuss and learn about the latest developments and advances in experimental design.
Experimental design workshops are also held during our annual group meetings to keep up to date with latest advancements.

Home Office

NON-TECHNICAL SUMMARY

163. Regulatory RNA mechanisms in germ and stem cells.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Germline, Developmental biology, epigenetics, RNA modifications, piRNA

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We are interested in reproductive cell (germ cell) development with a particular emphasis on the molecular mechanisms that underpin how chemical changes are made to gene DNA or chemical modification of the RNA produced from genes. We explore these topics predominantly in vivo and focus on RNA-based mechanisms that

regulate gene expression.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The objective here is to understand aspects of molecular biology that sustain reproductive cell (germ cell) development. The germ line is the lineage of cells that gives rise to the sperm and eggs cells that are responsible for the continuity of life and the genetic health for a given species. The germ line is passed from generation to generation and therefore merits special attention as it bears responsibility for the transmission of the genome and memory of modified gene expression patterns across generations. The understanding of how this lineage develops and how it is maintained throughout life bears importance to human health and socio-economic welfare given the dramatically increasing age of parenthood observed in Western societies and the associated age-related risks of infertility or birth defects.

What outputs do you think you will see at the end of this project?

We aim to identify the mechanism by which the small non-coding RNAs termed piRNAs recognize and silence jumping genes in male reproductive cells. We also aim to gain new insights into the function, mechanism and regulation of some of the RNA modification pathways in reproductive cells. These findings will give great insights into fundamental biological processes of gene regulation and reproductive cell development and will be published in high quality, peer-reviewed science journals and presented at international conferences. Furthermore, we hope to establish new collaborations based on our findings with experts in adjacent fields.

Who or what will benefit from these outputs, and how?

The benefits of understanding the mechanism by which the Piwi-interacting RNA (piRNA) pathway contributes to the establishment of epigenetic transposon silencing (Objective 1):

The function of germ line reprogramming is to reset genomic imprinting of the maternal and paternal chromosomes to that of the sex of the animal. Disrupted reprogramming results in sterility and defects in imprinting of specific parts of the chromosome have been associated with many human diseases exemplified by Prader-Willi or Angelman syndrome. Therefore, research on this topic offers benefits of understanding mechanisms that underpin mammalian development and genome stability.

The benefits of exploring the physiological importance and regulation of RNA modification in germ cell development (Objective 2):

RNA modifying pathways have been associated with human diseases or congenital disorders, such as cancer, obesity, infertility, underscoring the importance of these modifications for basic human development and physiology. Therefore, this aim delivers benefits that will advance knowledge on disease mechanisms as well as understanding of emerging facets of basic RNA molecular biology.

Given the majority of the work centres on basic germ cell and molecular biology, the work will be disseminated by two major routes. Firstly, the research will be presented to the respective communities at international scientific meetings. Secondly, the work will be published in peer-reviewed journals. Thus, the secondary benefits may therefore not only be developed in my own laboratory but also by the scientific community.

How will you look to maximise the outputs of this work?

The output of this work will primarily be published in open-access, peer-reviewed scientific journals as well as presented at international scientific conferences. After publication, data will be deposited in an open access digital repository. Published materials/reagents/mice will be available upon request to allow for collaboration and dissemination of new knowledge. Our data will also be safely stored to ensure their longevity and that they can easily be shared, uploaded or reanalysed by everyone.
Additionally, I aim to coordinate with our public relations team to make our results available to the wider public and understandable to the lay person.
Species and numbers of animals expected to be used

- Mice: 25,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

My laboratory is primarily interested in mammalian reproductive cell (germ cell) development and studies it from an RNA perspective. For this research, the use of animals is currently unavoidable as many facets of germ cell biology can only be studied in animals, such as mice, where these cells and developmental processes naturally occur. For example, the current knowledge does not allow us to properly culture mammalian germ cell cells in a dish – once plated into a dish they die or lose their potential, likely because of the missing tissue (micro-)environment present in vivo. Thus, current culture conditions do not allow us to test many key properties of germ cells. This necessitates the use of an animal model to study germ cell biology at the appropriate life stages.
Typically, what will be done to an animal used in your project?

Much of our work (greater than 90%) involves breeding, collection and analysis of tissues and a humane killing method only. In order to collect eggs for study, some animals need to be injected with hormones to induce superovulation which causes mild temporary discomfort but no lasting adverse effects. Animals primed with hormones for superovulation will be killed by a humane method of killing days after injection.
What are the expected impacts and/or adverse effects for the animals during your project?

We expect the observable characteristics (phenotypes) of mice used in this study to cause defective germ cell development or loss of germ cell. This renders mice infertile or sub-fertile and does not have other ill effects on mice that would cause suffering. Therefore, our work does not by default inflict suffering. Some animals need to be injected with hormones to induce superovulation, which cause mild transient discomfort and no lasting harm. Our work therefore causes minimal adverse effects to the animals.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Sub-threshold severity will apply to the majority of mice. Very few animals will suffer mild transient discomfort and no lasting harm due to injections required for superovulation.
What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The research of my laboratory focuses on the mammalian germ line, which makes the use of animals currently unavoidable as many facets of germ cell biology can only be studied in animals, such as mice, where these cells and developmental processes naturally occur. For example, the current knowledge does not allow us to properly culture mammalian reproductive cells in a dish – once plated into a dish they die or lose their potential, likely because of the missing tissue (micro-)environment present in vivo. Thus, current culture conditions do not allow us to test many key properties of reproductive cells. This necessitates the use of an animal model to study reproductive cell biology.

Which non-animal alternatives did you consider for use in this project?

We have considered and are making use of established cell lines as well as work on generating mammalian cell or tissue culture systems to study the RNA pathways and modifications. Furthermore, we have begun to investigate molecular and biochemical features of proteins of interest through in vitro analysis of purified proteins.

Why were they not suitable?

Unfortunately, in vitro mammalian tissue culture and germ cell systems that recapitulate reproductive cells development faithfully do not currently exist, nor do in vitro cultured foetal reproductive cells undergoing de novo DNA modification. None of the available systems provides the full complement of factors required for gene silencing or reproductive cells specific expression patterns of RNA modification pathways. Furthermore, the use of purified proteins only allows for the study of isolated biochemical functions that are independent of the larger, intricate complexes formed in vivo as the replication of complexes in vitro is currently impossible using recombinant purified proteins. This necessitates the use of an animal model to study RNA-based processes in germ cell development.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals used in each protocol is estimated based on my extensive previous experience and power calculations taking into account the number of experiments needed and number of mice per experiment required to obtain the appropriate statistical power.

The fact that the majority of experimentation is performed on either germ line, mice with the correct genotype but the opposite sex must be discarded, thus our project requires additional breeding over projects that analyse

mice independent of their sex. Additionally, some germ line observable traits are sensitive to the genetic background of the mouse line and thus newly generated mouse lines need to be crossed onto specific genetic backgrounds to make their analysis interpretable and scientifically sound.

According to our experience, we estimate for gene deletion and conditional genetics experiments that we require 100 experimental and 100 control animals per line per annum. Finally, for mice harbouring the correct genotype, we estimate that we would require 200 experimental mice required per year. Given we study several lines per year, we estimate that we would need to use 5,000 mice per year to obtain the necessary genotypes/sex which equates to 25,000 mice over the 5 years. These numbers include ca. 500 mice used on the superovulation protocol per year in order to collect sufficient amounts of oocytes for experimental use.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

As key means of achieving reduction we have and will continue to carefully design our experiments and mindfully estimate the number of animals required in order for the results of our experiments to be statistically convincing. To minimise the number of animals used in each experiment while optimizing the output of scientific data, we will make use of online tools (such as the NC3R's Experimental Design Assistant) and power calculations, calculations that allow us to predict the minimal number of animals to test to gain statistically significant information on a given phenotype. For example, in preliminary studies where we are trying to determine the most effective number of cells, or drug dosage to use, no more than 3 animals per group will be used when possible.

Importantly, experiments will be carefully planned to maximise the information obtained per animal and thus limit the subsequent use of additional animals. All methods requiring cells or tissue from animals will be carefully optimised in order to minimise the number of animal cells required. For example, we managed to reduce the number of foetal testis required for interaction screens by a factor of 5 in the last two years and likewise optimized input material required for RNA sequencing techniques.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

An important means of achieving reduction is to apply the most efficient breeding strategies. My group has extensive expertise with mouse colony management (i.e. monitoring large cohorts of mice) and we will strive to employ the best breeding schemes. We will replace breeders before their reproductive performance declines, and non-productive breeders will be replaced.

My group is fully aware of the ARRIVE guidelines and experimental design tools provided from NC3Rs. As such, we employ the optimal experimental designs to implement the 3Rs and optimise the number of mice for each experiment.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The majority of our work involves the use of genetically modified mice to test gene function in the germ line, protein tags to probe their molecular function, isolate and characterize populations of reproductive cells using marking strategies. Some mouse lines show phenotypes of defective reproductive cell development or loss of reproductive cells, which renders mice infertile or sub-fertile but does not have other ill effects on mice that cause suffering. Some animals (less than 10%) need to be injected with hormones to induce superovulation using well established procedures for the collection of eggs, which causes mild transient discomfort and no lasting harm. Much of our work (greater than 90%) therefore involves breeding, collection and analysis of tissues following a humane method of killing only. Thus, our work does not by default inflict suffering.

Why can't you use animals that are less sentient?

The tools that modify gene expression and genomic protein function evolve rapidly. This means that these modification pathways are found in very few other model organisms where there is a clear homologue in mammals. Thus, we are dependent on using mouse foetal testes as the only system available to understand the mammalian pathway. Likewise, my group specializes in studying the in vivo regulatory role of RNA modifications in mammals and thus we require the usage of mice as our model system.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To ensure technical competence, all staff will be supervised by the project license holder. Animals exhibiting any unexpected pain or suffering will be humanely culled. All procedures involving injections of substances are based on the scientific literature as well as extensive in-house experience and constantly refined to use the lowest dosage and frequency as possible, both to achieve our scientific goals and to minimise suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Our experimental designs are based on our long-term experience in this field (we have already refined many protocols and experimental designs) and peer-reviewed high-quality literature. We always seek to apply the most refined methods which are published in the field. We also base our knowledge on ample literature disseminated by NC3R and interactions with many expert colleagues and collaborators. We will always continue to refine our protocols based on the literature and knowledge exchanged with skilled collaborators and we are very proactive in this area.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Many advances in reproductive line research, including advances in mouse models, are disseminated during scientific conferences and seminars, which we frequently attend. Further, members of my group frequently attend 3Rs events organised by NC3R, and we continue to update our knowledge through the literature NC3R disseminates. We also have an extensive network of local and international collaborators who use similar state-of-the-art models, and by frequent exchange of information we will always stay informed about the best advances in the field. Given that our group is extremely committed to the implementation of the 3Rs, we will utilise any useful knowledge learned during these events and the literature to improve mouse procedures, apply cell culture models where possible, improve statistical methods, and minimise pain and suffering of our experimental mice.

Home Office

NON-TECHNICAL SUMMARY

164. Respiratory biology and pharmacology

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Respiratory, Therapeutics, Pharmacology, COPD, asthma

Animal types	Life stages
Mice	adult, aged
Rats	adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Understanding the biology of the respiratory system in experimental animal systems and using that knowledge to identify and develop novel treatments for human airway diseases

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Diseases of the respiratory system, such as asthma and chronic obstructive pulmonary disease (COPD), remain a significant cause of morbidity and mortality across the world. The World Health Organisation (WHO) estimates that 235 million children suffer from asthma and 6% of all deaths are caused by COPD. At the time of writing this application there is a global respiratory virus pandemic resulting in severe disease and high levels of suffering and loss of life. Understanding the mechanisms which result in respiratory disease enables us to design and develop new medicines which can increase the quality of life for patients.

What outputs do you think you will see at the end of this project?

We can reasonably expect that at the expiry of this licence we will have progressed at least three novel treatments for human airway diseases into early clinical testing. To get to this point we will have generated a great deal of novel information regarding the biology of the mechanisms involved and pharmacology of the novel treatments, and we would hope to have published where these data are not commercially sensitive.

Who or what will benefit from these outputs, and how?

We expect the new medicines we will discover and develop to have significant positive impacts on the lives of patients, their families and society as whole. These benefits may not be tangible until 10-15 years after initial discovery, so they are unlikely to be measurable during the lifetime of this licence. The new knowledge we will create and share will be of great use to other research groups in academia and in the pharmaceutical industry, and will enable other new medicines and treatments to be generated.

How will you look to maximise the outputs of this work?

We will share the new knowledge whenever we can through publications, conference posters and presentations. We have networks of academic collaborators with whom we share our findings and benefit from the discussion and validation of our findings. We are aware that there is a need to share negative findings as well as positive ones, and we will engage with others in the scientific community to understand if there may be ways to share non-commercially sensitive information that might reduce animal usage.

Species and numbers of animals expected to be used

- Mice: 4750
- Rats: 1750

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult and aged rats and mice are appropriate animals to study respiratory diseases as they have anatomically and physiologically relevant mammalian biology. Adult animals (>6 weeks old for mice, >8 weeks old for rats) are fully formed and can demonstrate representative tissue and immunological responses to inflammatory or other stimuli. Aged animals (>40 weeks in mice, >1 year in rats) may be used as we recognise that in some respiratory diseases (such as chronic obstructive pulmonary disease (COPD)) age itself may be a factor that limits the ability of the lung to repair or regenerate and we wish to understand mechanisms that could surmount this.

Typically, what will be done to an animal used in your project?

A typical study may involve 6 groups of 5 mice, which might be identified using a subcutaneous microchip after arrival, randomisation into cages and acclimation. At least 5 days after delivery animals they might receive a dose of a new treatment into the lungs in a very small volume of liquid (under anaesthesia) followed 1 hour later by a challenge to the lungs with a substance (again in a small volume) that will trigger inflammation (under anaesthesia). Animals would be returned to their home cage once recovered from anaesthesia, closely observed to ensure they are not exhibiting any unexpected side effects, checked again at the end of the working day and then left overnight. Animals are checked first thing in the morning and then at 24h after the lung challenge they would be anaesthetised before a final blood sample is taken. Animals are killed and their death confirmed before we rinse out the lung tissues to obtain a sample of cells.

What are the expected impacts and/or adverse effects for the animals during your project?

Inflammation of the airways is likely to be uncomfortable and animals may exhibit clinical signs of discomfort or illness such as loss of appetite, reduced grooming and reduced social interaction. Reduced food intake can lead to a body weight loss, and for this reason animals are weighed regularly (at least twice a week, daily of we see any changes in condition). Most of the studies we conduct are short (less than 3 days) although some experiments studying chronic airway remodelling or injury can last for 10 weeks or more.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice and rats:

We expect approximately 50% of animals to be classified as Moderate, the remaining 50% being Mild. This will depend on the specific studies but in many cases we will have negative (non-diseased) controls, positive controls (disease but given a known treatment) and animals treated with new experimental materials which we expect to reduce the disease and thereby reduce suffering.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Respiratory diseases arise through complex interactions of multiple biological, genetic and environmental factors. The structural cells of the lung tissue, the underlying vascular and nervous system components, and the immune cell populations all interact in ways which cannot yet be modelled with sufficient predictive power to replace animal studies, especially when novel mechanisms which are not fully understood are involved.

Which non-animal alternatives did you consider for use in this project?

Non animal systems are a key part of our research programmes, and animal studies are only considered when sufficient data are available to justify their use or in certain circumstances where the biology is novel and not characterised sufficiently to enable in vitro emulation.

Why were they not suitable?

The in vitro systems we do use are not complete representations of the lung in health or disease. Cell culture systems, even when they have some structural homology (such as organoid culture) do not have the complex biology seen in living organisms where multiple types of cell and structure combine.
Human tissue, when available, is not a complete surrogate as it lacks exposure of the lung cells to air (in submerged culture), blood circulation, innervation, immune cell trafficking and many other components.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Estimates are based on anticipated throughput of studies and using numbers typically used in previous experiments. Over a 5 year period usage may vary across protocols as the demand from as yet unknown research projects emerges. Rather than defaulting to maximise the estimates across all protocols I have kept number within the range of recent experience so it is possible that future amendment may be required.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Study designs are reviewed as part of our internal statistical review system to ensure that studies are appropriately constructed to account for biases and are sized to enable robust conclusions to be drawn.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Much work is conducted in vitro on cells and human tissue prior to human work, which reduces animal usage for preliminary experiments. Breeding of rats and non-genetically modified mice is performed for us at a commercial supplier which enables the maximum efficiency of animal usage for these animals. Genetically modified mice are not bred under this licence but are supplied under continuous use authority. The breeding schemes for these lines are closely monitored and for established lines with straightforward genetics (e.g. a single knockout or manipulation of a single locus) homozygous breeding is used to reduce wastage significantly (as 100% animals have the correct genotype rather than 25% for heterozygous breeding).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and

methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mouse and rat models which enable us to study the mechanisms involved in human lung diseases such as asthma and chronic obstructive lung disease (COPD), as well as complications such as viral exacerbations which can lead to hospitalisation and death for severely affected patients. We need to induce airway inflammation and cause changes to the lung tissues which reflect these human diseases, so it is likely that animals will experience respiratory changes and discomfort that cannot be avoided. However, as we are seeking to find and develop new medicines, many animals would be likely to have this suffering ameliorated by our novel treatments. We have selected airway models that are well tolerated and are expected to result in a moderate level of severity, which includes some clinical signs such as reduced food intake and lack of grooming. Where possible we will carry out studies using anaesthetised animals that can be immediately killed without recovery, to minimise suffering.

Why can't you use animals that are less sentient?

We are seeking to identify and develop treatments for human use and therefore although there may be some analogous systems in lower species such as flies, worms or fish we would not expect therapeutic agents which have been selected in vitro to work against human receptors or mediators to have the necessary efficacy. These lower species also do not reflect the mammalian and human immune systems and respiratory structures. This licence application does include a protocol for purely nonrecovery studies where all steps are performed under terminal anaesthesia.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals are closely monitored and are kept in modern purpose built facility with highly trained and motivated technical staff. We implement many systems to improve animal welfare, including nonaversive handling (i.e. not picking up by the tail), environmental enrichments such as tunnels, nesting materials and chew sticks, as well as cage floor level access to food for animals displaying reduced activity or mobility.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Best practice, for example the use analgesics after surgical procedures will be employed to minimise suffering.

Publications such as the Handbook of Laboratory Animal Care and Welfare (Wolfenson and Lloyd), and the LASA best practice guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I am in regular contact with other in vivo scientists and teams of animal welfare specialists within and outside my organisation. I also convene regular 3Rs events where we meet with local and regional partners to share our best practices.

Home Office

NON-TECHNICAL SUMMARY

165. Responsiveness of adult neural stem cells to diet-induced obesity and exercise

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how diet and exercise regulate neural cell networks in the brain, which control feeding behaviour. Specifically, we will study cellular and molecular changes in adult neural stem cells and their cell progeny in the neurogenic niches of the adult brain.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In order to develop new procedures to combat the development of obesity, we need to elucidate how diet and exercise affects the regulatory centres of the brain. Most of the cellular and molecular components of these centres are understood, except for adult neural stem cells. These cells can generate newborn nerve cells in the brain, which contribute to critical functions such as memory or feeding control. We need to understand how the ability of stem cells to generate new nerve cells is affected by diet-induced obesity and if the negative effects of obesity can be rescued by exercise. If we can identify, which cell processes and what molecular factors in the stem cells are affected by the diet and exercise, we can develop new approaches on how to control hunger sensation and development of obesity.

What outputs do you think you will see at the end of this project?

Our goal is to understand how obesity-inducing diet and physical exercise influence the brain. Particularly, we aim to gain insights into how obesity affects the resident neural stem cells, which can generate new neurons in the adult mammalian brain. We will investigate how obesity affects the stem cells and new neurons and how these new neurons may regulate the sensation of hunger and appetite, which are the driving forces behind development of obesity. Also, we will determine how prenatal exposure to obesity-inducing diet may affect adult brain. Finally, we will be testing novel anti-obesity drugs, which may decrease the sensation of hunger by acting on the new neurons. Novel findings from the project will lead to better understanding of the relationship between obesity and brain and provide pre-clinical testing for anti-obesity therapies. The findings will lead to peer-reviewed publications and new grant funding.

Who or what will benefit from these outputs, and how?

In the short-term, outputs such as new information and experimental models will benefit the neural stem cell and neuroscience research communities. In the long term, the research findings will have impact on anti-obesity therapies and approaches in direct and indirect ways. The direct ways include better characterisation of novel anti-obesity compounds. The indirect impact consists of better understanding of genetic and cellular mechanisms in the brain that are regulating hunger sensation and are responsive to obesity.

How will you look to maximise the outputs of this work?

All knowledge generated during this project will be disseminated as peer-reviewed publications and presented at national/international scientific meetings. Data will be shared via open access journals and repositories. We will continue to actively engage with the general public via events for children or adults within the University or in local communities. Progress and successes in key milestones will be released via University research pages, newsletters and social media.

Species and numbers of animals expected to be used

- Mice: 9000 (both males and females)

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of

the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will study the effects of environmental interventions, such as diet-induced obesity or anti-obesity treatment, on resident neural stem cells in the brain. We will use mice as the most suitable experimental animal model for studying obesity for the following reasons: there is a large body of knowledge on the physiology, histology and molecular biology of the mouse's CNS; there are a wide range of existing genetically modified lines, a number of which are directly relevant and amenable to our studies; the relatively short life-cycle and high fecundity of mice is advantageous for genetic studies; and mice are generally regarded as being of lower sentience compared to other mammals such as the primates. In order to model the impact of obesity on brain, we induce obesity either in adult animals or expose pregnant females to obesity-inducing diets or anti-obesity compounds. This will allow us to study the effects of prenatal exposure to obesity-inducing diets and/or anti-obesity compounds on adult brain. Diet-induced obesity and actions of anti-obesity drugs act on the brain in a complex way, which cannot be simulated only in cell cultures.

Typically, what will be done to an animal used in your project?

Typically, we induce conditional gene expression in adult mice (e.g., 6-8 week old) using inducible-Cre recombinases and under the control of a tissue-specific genetic elements (called promoters), e.g., tamoxifen induced expression of the green fluorescent protein or removal of a specific gene in the adult neural stem cells. The animals will receive several injections of tamoxifen to induce transgene expression. Induced animals are aged to a defined end point (e.g., 14 or 28 days) before being killed by a Schedule I method or by transcardial perfusion under total anaesthesia to obtain tissue for further studies. Alternatively, we assign animals to a controlled feeding programme (e.g., high fat diet for 3 months), and/or to a voluntary physical exercise on running wheels. In addition, some animals will be administered previously tested anti-obesity compounds by repeated injections after the exposure to diet-induced obesity. Exposed animals are aged to a defined end point (e.g. 14 or 28 days) before
being killed by a Schedule I method by transcardial perfusion under total anaesthesia to obtain tissue for further studies.

What are the expected impacts and/or adverse effects for the animals during your project?

We expect that obesity-inducing diets will generate obesity, which can be accompanied by increased tissue inflammation or reduced physical activity, however, without causing any painful experience. Animals may experience diet-induced obesity for several months. The genetically altered animals do not show any overall harmful phenotype in the general health of behaviour of the animals.
Administration of substances such as tamoxifen or anti-obesity compounds by injections will cause a brief discomfort without any lasting harm.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Typically, experimental animals will experience only mild severity. Rarely (<1/20), animals may develop more extreme forms of obesity, which may be classified in the moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The complex regulatory mechanism of the brain and the interactions between diet and different organs cannot be replaced by in vitro models. There are reductionist cell-culture models, however, they can only mimic limited biological processes on the cell level but not on the tissue or organ levels. We will be using certain primary cell-culture models as a part of the reduction strategy; however, these are also derived from animals. The available *in vitro* models do not allow to model the complexity and interconnectivity of organs and tissues that occurs in animals. The very development of diet-induced obesity requires food intake and thus requires experimental animals. Diet-induced obesity in mice accurately model the development of obesity in humans. Mice represent a sensible compromise between mammalian model close to human physiology and more sentient mammalian species such as primates.

Which non-animal alternatives did you consider for use in this project?

We have considered in vitro brain organoids or cell cultures of neural stem cells.

Why were they not suitable?

In vitro models cannot recreate all the complex aspects of diet-induced obesity or exercise that occur in actual animals. Diet or exercise do not exclusively target discrete brain cells, or brain alone, but exert a very complex influence on the whole body, including cardiovascular and endocrine systems, whole body metabolism and immune system. All these complex effects, many of which are unpredictable, cannot be modelled in vitro, using only selected cell populations. Even the most sophisticated ex vivo brain organoid models do not robustly recapitulate healthy normal tissue and completely lack the feedback and regulatory mechanisms from the peripheral organs such as the intestine or the spleen.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

NC3Rs Experimental Design Assistant and Power Calculations. Effect size (m1-m2) and Variance (S.D.) were calculated using current data.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

All calculations were made using the NC3Rs Experimental Design Assistant and current data.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will breed heterozygous animals in trios to increase efficiency. On average, 50% of the offspring of genetically altered animals will have the desired phenotype. As a reduction strategy, we will be using primary cell-cultures derived from neural stem cells of the brain. These cell cultures can be propagated and utilized for parallel experiments such as testing different concentrations of drugs or studying simplified cell behaviours in

vitro. When possible, we will collaborate with other stem cell laboratories at the university or abroad to share animal-derived tissue. For example, we have arranged that animals from our experiments will be used for harvesting intestinal tissue by a collaborator lab, while we will be harvesting the brain. Vice versa, the collaborator's lab will provide their animals (used for isolation of intestines) for brain isolation. To reduce the overall number of used animals, we will use factorial experimental design as well as the power analysis. With anti-obesity compounds, we will use pilot studies to gauge efficacy (e.g., 2-4 animals).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The basic paradigm in this project involves exposure of animals to obesity-inducing diets, exercise or anti-obesity compounds. The animals will have free access to high-fat diet, which they consume voluntarily. The diet induced mild to moderate obesity by increasing caloric intake in the animals. The health of the animals will be continuously monitored and in case of any severe health effect, the high-fat diet will be replaced by the regular, healthy diet. Selected animals will be exposed to voluntary exercise, which improves their welfare and health. A proportion of mice will be administered chemicals that allow tracing of specific cell populations. These drugs are well tolerated and are accompanied by sub-threshold symptoms. A proportion of mice will be administered exogenous substances (known as xenobiotics) that can contribute to the development of obesity. Only classes of substances that have been used previously in animal studies will be used. Pilot experiments will always be performed where a new genotype/drug combination is being studied, with a decreasing dose regime applied on single mice, starting below established maximum tolerated doses, to minimise mouse numbers. Some animals may be examined by behavioural tests. As a part of the refinement effort, we will use most modern alternatives of the behavioural tests, which reduce the stress of animals (e.g. using paddling maze instead of Morris water maze). Finally, some animals will be administered selected anti-obesity drugs, which improve the long-term animal health by reducing the weight gain. Injection of these drugs will cause very brief discomfort, however, without long-lasting harm.

We will use genetically altered mouse models and site-specific Cre-lox technology that allows temporal and inducible expression of reporter fluorescence proteins or deletion of genes in specific cell types. This refinement will minimize any possible detrimental impact of transgenes on the animals. All genetically altered lines will carry conditional alleles; therefore, only mice that carry site-specific recombinase and the conditional allele will express the reporter, but only after induction of genetic recombination by tamoxifen. We will only use genetically altered animals appropriate to our objectives (i.e., targeting specific, well-defined cell populations in a tissue-dependent manner) to ensure that the work carried out is accurate. After the experiment, the animals will be killed to collect their tissue for histological or physiological analyses.

Why can't you use animals that are less sentient?

Mice represent the most suitable experimental animal model of obesity and stem cells for the following reasons. First, there is a large body of knowledge on the physiology, dietology and stem cell biology of the mouse central nervous system. Second, there is a wide range of genetically modified lines, which allows technical flexibility and refinement. Third, mice are regarded as being of lower sentience compared to other mammals with digestive tracts closer to humans such as pigs or with nervous system like humans such as primates. Because we will study the effects of environmental interventions such as altered diets on adult brain, we cannot use more

immature or anaesthetised animals. Nevertheless, we will ensure that the best animal welfare possible is maintained. The experimental mice will experience only limited discomfort during the injections. The exposure to obesity-inducing diets is comparable to health and welfare risks that majority of people in the Western society undergo voluntarily. Selected mice will have access to running wheels as a component of the experimental design, which will bring about the positive effects of environmental enrichment to improve the animal welfare.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinements will include frequent monitoring of induced animals, post-operative or anaesthetised animals, or animals on altered diets. Exogenous substances/agents will be administered at the minimum dose, as determined using NC3Rs guidelines for best practice and dosing regimens and following consultation with our collaborators. All procedures will be carried out by trained and experienced staff with the support of experienced animal facility staff/NACWOs/NVS (e.g., application of anaesthesia, analgesics, surgical techniques). Assessment of severity of clinical signs will be based on Clinical Signs Health Check Score Sheet:

Parameter	Animal ID	Score	Date/Time	Date/Time
Appearance	Normal	0		
	General lack of groomin	1		
	Staring coat, ocular and nasal discharge	2		
	Piloerection, hunched u	3		
Food and water intake	Normal	0		
	5% - 15% weight los	1		
	15% - 20% weight los	2		
	Over 20% weight los	3		
Pregnant females (2nd half of pregnancy)	Normal	0		
	Reddening and swelling of vulv	1		
	> 5% weight los	2		
	Vulvar bleeding or > 10% weight los	3		
Natural behaviour	Normal	0		
	Minor changes e.g. lack of nes	1		
	Less mobile and alert, isolatec	2		
	Vocalisation, self-mutilation, restless	3		
Provoked behaviour	Normal	0		
	Minor depression or exaggerated respons	1		
	Moderate change in expected behaviou	2		
	Reacts violently or very weak and precomatos	3		
Movement	Normal	0		
	Partial paresis of any limbs	1		
	Paresis of any limbs	2		
	Paralysis or partial paralysis of any limbs	3		
	TOTAL:			

0-3 = Norma
4-8 = Monitor carefully, consider analgesic
9-12 = Provide relief, observe regularly. Seek advice of NACWO and/or NV
For mild protocols: score of 10 or greater = Kill by a Sch 1 metho
For moderate protocols: score of 12 or greater = Kill by a Sch 1 metho

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

This project will be carried out in collaboration with national and international researchers, who are experienced, have excellent track records and are world renowned leaders in stem cell biology, obesity and the use of

genetically altered mouse models.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Engage with NC3Rs representatives on a regular basis via local workshops/meetings, newsletters and social media.

Home Office

NON-TECHNICAL SUMMARY

166. Robustness of molecular programs driving cellular differentiation

Project duration

5 years 0 months
Project purpose

- (a) Basic research **Key**

words

cellular differentiation, embryogenesis, haematopoiesis, robustness, gene regulation

Animal types	Life stages
Zebra fish	embryo, adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim is to understand how individual gene regulators contribute to the robustness of cellular differentiation during zebrafish blood development.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our body consists of numerous different cell types that all fulfil specific functions. Cellular differentiation is the process by which these mature cells arise from immature precursor cells. While all precursor cells carry the same genetic information, each of them makes differential use of this information as it gives rise to a specific cell type. The gene regulatory network that is hard-wired in our genome determines the differentiation options of the precursor cells. It defines the target genes that a particular regulator can activate. Which part of the network is activated in a particular cell depends on prior gene expression (intrinsic factors) and on influences from outside the cells (extrinsic factors). As a cell differentiates it moves from one regulatory state to another. As it enters a new state, the cell not only activates a new set of genes, it also extinguishes the previous state and suppresses alternative options. Gene activators are needed to push the cell forward while gene repressors prevent cells from slipping back or taking alternative molecular trajectories. Together, they confer robustness to cellular differentiation and determine the pace of the process. Failure to establish or maintain robustness can lead to cellular deficiencies and cancer. Therefore, understanding how robustness is established and maintained is vitally important. In the short run, the project will provide a deeper understanding of the molecular programming of cellular differentiation. In the long run, patients may benefit from the basic knowledge generated during this project. It is reasonable to assume that the personalised medicine of the future will focus heavily on disease prevention. It will try to weigh the impact of genomic sequence variations
on the future health of the patient. It will also want to judge the suitability of donor cells used in regenerative medicine. This project will contribute to the development of a knowledge base that is needed to make such evaluations.

What outputs do you think you will see at the end of this project?

The idea of the project is to gain a better understanding of the molecular programme that drives differentiation in various cell types and the role that particular molecular players play in this process. We will learn more about the way that these players interact to ensure the robustness of the differentiation process. Because some of these molecular players are known to be involved in various types of cancers our data will also provide new insights into pathways that lead to cancer. The datasets that we will generate will be deposited in public databases. The conclusions drawn from the papers will be presented at scientific conferences and will be published in peer-reviewed journals. The reagents and genetically modified fish lines will be made available to other researchers in the field.

Who or what will benefit from these outputs, and how?

Our findings will be interesting to developmental biologists who want to gain a better understanding of cellular differentiation. Our work will focus mainly on blood cell development. Thus, scientists and clinicians working in haematology will benefit from our findings. Our data will also be interesting to scientists and clinicians in the field of oncology because many of the genes that we work on have been implicated in cancer. All beneficiaries will have access to the datasets that we will deposit in public databases. Reagents and genetically modified fish will be available on request. All publications on original data will be accessible through open access.

How will you look to maximise the outputs of this work?

Data from the study will be published in peer-reviewed, high profile, scientific journals and presented at local, national and international conferences. Data will also be publicised through the establishment's web page and in public engagement exercises. This study does not propose to develop new techniques. Thus, unsuccessful experimental approaches are not expected. It is, however, possible that the results reject our hypotheses. Such results will be discussed as part of a paper that supports a different interpretation.

Species and numbers of animals expected to be used

- Zebra fish: We estimate that we need to maintain about 19,600 adult fish in our breeding programme: 1,600 for the generation of new lines and 18,000 for the maintenance of old lines.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We want to study the differentiation of blood cells in their natural environment and shed light on the roles that molecular players, like the Gfi1/1b repressors, the epigenetic regulators Tet1-3 and two newly identified long-noncoding RNAs play in this process. We are using zebrafish embryos as our model system. Most experiments will be performed on embryos up to day 5, the point when they become freefeeding and fall under the regulations of ASPA.

Typically, what will be done to an animal used in your project?

To generate transgenic and genetically modified zebrafish, early stage embryos will be injected with DNA, RNA or proteins. Injected embryos may be anaesthetised to assess their phenotypes, i.e. to see whether they express the reporter transgene and whether they develop a normal swim bladder. Phenotypically normal zebrafish will be allowed to grow beyond the time point when they become freefeeding. This is when they become protected by Animal (Scientific Procedures) Act 1986 (ASPA). We expect to raise up to 1,600 fish over a period of 5 years to generate the genetically modified lines that we need for our experiments. Once the injected fish reach sexual maturity we will check whether they carry the transgene/genetic modification in cells of their germ line. For this purpose, we will cross the fish and collect embryos that we will examine for the presence of the transgene/genetic modification. Progeny that carry the genetic modification will be raised to adulthood. They will found a new line of genetically modified zebrafish. Newly generated and previously established lines are maintained on the breeding and maintenance programme. Fish on this programme may be anaesthetised and finclipped for genotyping purposes at embryonic or adult stages. They may also be anaesthetised at larval stages to assess their phenotypes, e.g. study the expression of a transgene or verify their normal phenotypes. Most fish will carry a single copy of the transgene or modified gene allele, i.e. will be heterozygous carriers. To study the impact of a particular genetic modification on cellular development, heterozygous carriers are incrossed to generate homozygous carriers, i.e. embryos with two copies of the modified allele. These embryos/larvae will be subject to experimentation up to day 5. Experience tells us that we need 18,000 fish on the breeding and maintenance programme to have enough breeding pairs of males and females for the lines that we need to keep over the 5 years of this licence. In cases, where homozygous larvae or larvae with new combinations of mutant alleles are phenotypically normal on day 5, homozygous larvae may enter the breeding and maintenance programme and be raised to adulthood.

What are the expected impacts and/or adverse effects for the animals during your project?

Injection of DNA, RNA and protein may cause embryos to show abnormal phenotypes. Likewise, homozygous carriers of mutant alleles and carriers of new combinations of mutant alleles may display abnormal phenotypes. Larvae that show abnormalities will not be allowed to develop beyond the freefeeding stage. Larvae that are normal on day 5 may be raised to adulthood. In rare cases, such larvae may develop persistent abnormalities later. These animals will then be killed by an S1 procedure. In very rare cases, fin clips used for genotyping may lead to persistent and/or significant infections. These animals will also be killed by an S1 procedure. Animals may also transiently experience deteriorating water quality when being kept in isolation and off the recirculating water system. To minimise negative effects, animals will be kept off the recirculating water system for a

maximum of 24 hours.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most animals (about 66%) will show sub-threshold symptoms. Experience tells us that up to a third of animals will be fin-clipped for genotyping. These will experience mild and transient symptoms (about 33%). Rarely (<1%), animals (like homozygous carriers of a modified gene allele or carriers of new combinations of modified gene alleles) that are phenotypically normal on day 5, may develop more severe, i.e. moderate, symptoms at a later time point. Persistent and/or significant infections cause symptoms of moderate severity. These are very rare (<1% each).

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In vertebrates, cell fate specification and cell differentiation heavily rely on the cell's interactions with its neighbours in the embryo.

Which non-animal alternatives did you consider for use in this project?

One may argue that cellular differentiation could be studied in cell culture experiments.

Why were they not suitable?

However, faithful reproduction of in vivo cell-to-cell interactions is extremely difficult in cell cultures, if at all possible. Furthermore, the generation of cells with the correct experimental and control genotypes is not feasible.

By focussing our work on early stage zebrafish embryos, we are achieving what is considered to be a partial replacement of animal work. Juvenile and adult stage zebrafish will mainly be used for breeding purposes. Occasionally, they will also be used as tissue sources after terminal anaesthesia.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

On the breeding programme, we maintain existing genetically modified lines. We aim to maintain a good balance of males and females for each of the modifications to be able to derive homozygous embryos from incrosses of heterozygous male and female parents. Unfortunately, sex determination in domesticated zebrafish lines does not involve sex chromosomes. Instead, it is polygenic and complex, and can be subject to environmental influences, in particular temperature, food availability and stocking density (Kossack and Draper, 2019, Curr. Top. Dev. Biol., 134, 119-149). Thus, female/male ratios in offspring can vary widely from batch to batch, even if the parent fish involved are the same. This makes power calculations and statistical modelling impossible and forces us to rely on experience. On this licence, we want to maintain 60 different established lines of genetically altered fish over 5 years. To have enough breeding pairs of males and females, experience tells us that having 60 fish per line in our aquarium at any time is sufficient to achieve this goal. The overall number of fish required is therefore: 60 lines x 60 fish x 5 years = 18,000 fish.
Tol2 transgenesis and CRISPR/Cas9 targeting experiments are performed to achieve a particular goal, the generation of a genetically modified line. Success rates vary considerably in goal-directed experiments and can, therefore, not be modelled statistically using power calculations (Dell et al., 2002, ILAR J. 43 (4), 207-213). There are 2 sources of variability in our experiments. Firstly, the success rate varies with the type of genetic modification that we want to generate. For example, the generation of a simple small insertion/deletion (indel) mutation at a single CRISPR/Cas9 target site is easier to achieve than the deletion of a 10 kb DNA fragment between two target sites. Secondly, germline transmission can vary considerably. Successfully injected embryos are mosaic for the modification, and germline transmission is difficult to predict. Because we cannot use statistical modelling, we rely on previous experience to calculate animal numbers. Assuming that we will (a) generate 20 new transgenic or mutant lines over 5 years and (b) grow up an average of 80 injected larvae per experiment to find transgenic/mutant founders, we will need 1,600 animals overall.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The NC3Rs experimental design assistant is used. Furthermore, advice is taken from local statistics experts.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In transgenesis and genome editing experiments, we aim to minimise the number of injected animals that we grow beyond the free-feeding stage. We will achieve that by carefully assessing and optimising the success rate of transgene integration and genome targeting in the soma of injected siblings at embryonic stages. Whether the modification is also present in the germline cannot be judged at this stage.
We will use pilot studies to grow up animals with new combinations of genetic modifications that could reasonable be assumed to cause deficiencies. Initially, 5 experimental and 5 control embryos will be grown up in parallel.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

To be able to transfer insights gained in our experiments to the situation in humans we need to use a vertebrate model organism. Zebrafish is already the least sentient vertebrate model organism that is regularly used in project like ours.

Why can't you use animals that are less sentient?

Most of our experiments will be performed on the most immature life stages, i.e. up to 5 days post fertilisation. These stages are not covered by ASPA. Fish larvae that display obvious abnormal phenotypes on day 5 will not be raised beyond day 5. Adults may serve as source of tissues after terminal anaesthesia.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We previously optimised our fin clipping procedure. We carefully titrate up the anaesthetic until we are convinced that the correct anaesthetic depth is reached before the fish is removed from the water and fin-clipped.

We have considered to use low-dose lidocaine immersion to aid perioperative analgesia (Schroeder and Sneddon (2018) Applied Animal Behaviour Science 187 (2017) 93–102). Unfortunately, the data presented in this paper provide insufficient experimental evidence of its efficacy. On the basis of these data, we cannot justify the exposure of our fish to an additional treatment. We will keep an eye on further developments and reconsider the use of analgesia when better evidence becomes available.

We recently tried to use the published skin swabbing protocol (https://www.nc3rs.org.uk/quantifyingpotential-skin-swabbing-refinement-dna-sampling-laboratory-fish) in parallel to fin-clipping to collect genetic material from 10 animals after S1. Performing the experiment after S1 allowed us to swab extensively, giving us the best chance to get an optimal yield of cells. DNA was prepared and PCR amplified from the swab and fin-clip samples. While the fin-clip samples gave us unambiguous results for 10/10 samples, the swab experiment yielded clear data in only 5/10 cases. This result is not satisfactory. We are happy to keep reviewing alternative methods.

Earlier this year, we introduced two changes to reduce the time it takes to genotype the fish and, thus, the time that fin-clipped fish need to be kept in isolation. Firstly, we now keep fin-clipped fish in a fish grid tank rather than in individual tanks. This reduces the time we spend on labelling and handling tanks. It allows us to move to the DNA extraction more quickly. The fish may also appreciate seeing their siblings in neighbouring compartments. Secondly, we are in the process of speeding up our DNA extraction procedure by replacing an overnight incubation with a 30 min procedure. As this procedure yields lower quality DNA we are currently testing the extracted DNA in all of the different genotyping PCR experiments. We have seen that the DNA is good enough for our most reliable PCR assays. Others still need to be tested.

Larvae that carry a novel combination of gene alleles will initially be raised in small numbers (5 experimental and 5 control larvae) in pilot experiments. These will typically be monitored twice daily be the personal animal holder.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The most up-to-date guidance on zebrafish housing and husbandry was published in July last year. The paper by Aleström et al summarises recommendations from a working group that consisted of members of the European Society for Fish Models in Biology and Medicine [EUFishBioMed] and the Federation of European Laboratory Animal Science Association [FELASA]. Basic methods on zebrafish embryo handling are described in the Zebrafish book that is published online on the zfin.org web page (http://zfin.org/zf_info/zfbook/zfbk.html). We use the CRISPR-Scan track on the UCSC genome browser to identify suitable, high efficiency targets for CRISPR/Cas9 (Moreno-Mateos et al., 2015). CRISPR/Cas9 experiments follow the protocol published Hwang and colleagues (2013). We have replaced Cas9 mRNA with Cas9 protein injection to achieve gene editing at earlier stages to reduce mosaicism in the injected embryos and increase the chance of germline transmission. These embryo injections follow standard procedures. The fin-clips and DNA extractions are performed as published by Wilkinson and colleagues (2013).

References:

Aleström et al., 2019, Zebrafish: Housing and Husbandry Recommendations, Laboratory Animals, 54 (3), 213-224. https://doi.org/10.1177/0023677219869037

Hwang et al., 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature Biotech. 31 (3) 227-229 https://www.nature.com/articles/nbt.2501.
Moreno-Mateos et al., 2015. CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo. Nature Methods 12 (10), 982-988. http://www.nature.com/doifinder/10.1038/nmeth.3543.

Wilkinson et al., 2013, A method for high-throughput PCR-based genotyping of larval zebrafish tail biopsies. Biotechniques 55 (6), 314-316. https://www.future-science.com/doi/10.2144/000114116.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Information and guidance published on nc3rs.org.uk, felasa.eu, zfin.org and eufishbiomed.eu are very useful. We also follow novel developments that are presented at national and international zebrafish meetings. The 3-minute-3Rs podcasts published on the NC3Rs web page are also a great source for new ideas, as is the information on the F1000 NC3Rs and the EURL ECVAM web pages.

Home Office

NON-TECHNICAL SUMMARY

167.Rodent models of heart failure progression

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cardiac hypertrophy, Heart failure, Trans-aortic constriction, Sumoylation

Animal types	Life stages
Mice	adult
Rats	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this project is to generate rat and mouse models of heart wall thickening and heart failure using surgical techniques to constrict the major blood vessel that exits the heart (i.e. transverse aortic constriction (TAC) procedures). We aim to use these models to better understand the molecular processes underlying the progression of heart disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The incidence of chronic heart failure in the UK is increasing, however the underlying causes are not fully understood, and more effective treatments are necessary.

This project will allow us to better understand the molecular genetic processes underlying heart failure development and aid in the identification of novel therapeutic targets.

What outputs do you think you will see at the end of this project?

Outputs from this project will include increased knowledge in the field of heart disease and identification of novel therapeutic targets.

The results of the project will be presented at scientific conferences and will be written up and published in peer-reviewed scientific journals. The data generated will also be used to support future grant funding applications.

The unique KO rat model, and/or stored biological samples generated from these studies will be made available to other scientists in the field, on request.

Who or what will benefit from these outputs, and how?

The expected benefits arising from this project are multi-fold and will be in relation to both the advancement of scientific knowledge (short-medium term) and further to potential clinical translation of novel therapies (longer term).

Molecular investigations of the target genes and proteins identified in our studies will lead to a better understanding of the mechanisms responsible for development of changes in heart wall thickness (cardiac hypertrophy) and heart failure. This novel information will allow us to develop new therapeutic

strategies to prevent or reverse the underlying pathophysiological processes, which are suitable for clinical application.

Specifically, utilisation of the TAC procedure in our unique rat models will allow us to confirm the role of target genes in cardiac hypertrophy and heart failure progression and will provide a platform for investigating the underlying pathogenic processes and development of novel therapeutic interventions.

The TAC model in the mouse will be used to examine the impact of specific protein modifications (sumoylation)that are critical for normal heart function. It is hypothesised that the sumoylation process is a natural protection mechanism by which cardiac proteins can be stabilised during heart failure. Confirmation of this process would be of enormous benefit for future clinical applications e.g. therapeutic strategies and screening.

These outputs will be of benefit not only to my own research group and my clinical and basic science colleagues at my research institution, but also to my network of national and international collaborators and to other researchers in this field, through dissemination of the results in published papers and scientific conferences. Information from these pre-clinical studies will inform future clinical studies, where the ultimate beneficiaries will be heart disease patients who will receive improved diagnosis and treatment.

How will you look to maximise the outputs of this work?

The results from this project will be disseminated to the cardiovascular research scientific community through presentation at national and international scientific conferences, publication in peer reviewed scientific journals and through our local and international collaborative network.

Dissemination of data from studies with negative or neutral outcomes will still be possible since publication of neutral and negative studies is now being actively encouraged by some journal editors.

Species and numbers of animals expected to be used

- Mice: 200
- Rats: 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

In this project we will use juvenile and adult rats and adult mice. These models have been carefully selected based on their unique genetic profiles. Some of the animals we use will have already established (spontaneous) hypertension. These animal models will allow us to determine the direct contribution of specific genes and proteins to the progression of heart disease and heart failure.

Previous studies with our rat model of hypertension have shown contrasting levels of expression of our gene of interest during early and adult stages, therefore we aim to study the impact of this differential gene expression on cardiac hypertrophy and heart failure development using both juvenile and adult rats.

Typically, what will be done to an animal used in your project?

During this project we will generate reproducible models of cardiac hypertrophy and heart failure in rats and mice in order to mimic the equivalent human disease. We will examine the mechanisms of heart disease progression in control animals and in animals which have been genetically modified to alter specific genes or proteins that may play an important role in the disease process.

The transverse aortic constriction (TAC) surgical procedure (i.e. permanent constriction of the major blood vessel from the heart) will initially cause an increase in blood pressure and heart size, which are not expected to result in any adverse clinical signs. However, over time the increased load on the heart, as a result of continued elevated blood pressure, will become detrimental and the heart will begin to lose its ability to function normally. We will monitor these structural and functional changes in the heart by echocardiography repeated at regular intervals during the pre- and post-surgery period. Echocardiography is a non-invasive method requiring brief anaesthesia for imaging purposes. Blood pressure will be monitored by non-invasive means on a weekly basis. Typically, animals will remain on procedure for up to 12 weeks (with a maximum limit of 16 weeks post-surgery). Any animal displaying signs of heart failure will be promptly and humanely killed.

During these TAC studies we may include optional steps where diet is modified, drugs are delivered, or expression of specific genes is altered in order to modify the heart disease processes. This will allow us to investigate potential therapeutic strategies. These studies may involve collection of blood and urine samples at regular interval during the pre- and post-surgery period.

What are the expected impacts and/or adverse effects for the animals during your project?

The TAC surgical procedure will initially cause an increase in blood pressure and heart size, which are not expected to result in any adverse clinical signs. However, over time the increased load on the heart, as a result of continued elevated blood pressure, will become detrimental and the heart will begin to lose its ability to function normally. All animals undergoing TAC surgery will be carefully monitored on a daily basis and any animal displaying signs of heart failure will be promptly and humanely killed.

Pain (100%). All animals will experience pain as a result of the surgical procedure. Analgesics will be given in consultation with the NVS for as long as necessary.

Anaesthetic death (1%).

Weight loss from repeated anaesthesia (1%).

Decreased palatability (1%) and weight loss (1%) as a result of dietary manipulation. Although the special diets are not expected to produce any major short-term weight loss or adverse clinical effects in rats or mice (other than the desired cardiovascular modification), palatability issues may result in gradual weight loss over longer intervention periods (e.g. 2 weeks or longer). Body weight will be

monitored at regular (typically twice weekly) intervals. Body condition scoring will be implemented if palatability issues are anticipated for specific diets.

We may use modified viruses to alter gene expression in our animal models. Acute toxicity to the maximum virus dose in our in-house colony of rats (5%). Sensitivity may be increased in rats from external sources (i.e. bought in from commercial suppliers). Acute toxicity is not anticipated in mice.

Sudden death due to stroke in the hypertensive stroke-prone rat model (6%)

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The TAC procedure is classed as moderate. 100% of animals will undergo either TAC or sham surgical procedure.

An improved (minimally invasive) method of transverse aortic constriction will be used in order to limit surgical complications.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The nature and complexity of the heart disease process makes finding alternatives to live animal models extremely difficult. Although in-vitro models are available to examine molecular mechanisms underlying heart cell size responses, there are limitations associated with interpretation of results from these models. These cells are mainly derived from new-born animals and therefore results may not be predictive of the cellular response in-vivo in the adult heart. Usually, a single cell type will be grown in isolation (e.g. cardiomyocytes, cardiofibroblasts), whereas in the in-vivo situation, different cell types develop together and function as units. Also, in-vitro systems have no blood supply, whereas in-vivo the circulation can contribute to the heart disease process by providing a pathway for disease causing molecules between the heart and the rest of the body. Therefore, it is important to understand the disease mechanisms in intact organisms, which show similar cardiovascular physiology to humans.

Which non-animal alternatives did you consider for use in this project?

Where possible we use non-sentient alternatives to live animals. For example, cell culture has replaced the use of animals in some of our functional analysis studies of candidate genes and gene networks and protein/protein interaction studies. Furthermore, we use cell culture to develop and optimise the virus gene delivery system in our gene transfer procedures before in-vivo testing is carried out.

To simulate some of the greater complexity of the whole heart, another consideration is the use of an organ-on-a-chip (OOC), which is a multi-channel 3-D microfluidic cell culture chip that can mimic the functions of human organs. This technology can provide a more detailed and more physiological approach than cell culture studies based on a single cell type. Whilst heart-on-a-chip systems are routinely used for drug efficacy screening, appropriate in-vitro model systems of human heart failure that fully recapitulate the essential changes and cardiac mechanisms seen in the disease are still in the developmental stage.

Human heart cells are difficult to obtain and can only be maintained in culture for a short period of time and are therefore not suitable as a robust testing model.

Why were they not suitable?

Although complementary cell-based assays can provide important detailed information on intracellular mechanisms and will be used alongside our rat and mouse studies, they cannot fully replace the use of live animals for investigation of heart disease progression where an intact cardiovascular system is necessary.

There are limitations associated with interpretation of results from in-vitro models. The heart cells used in these in-vitro models are mainly derived from new-born animals and therefore results may not be predictive of the cellular response in-vivo in the adult heart. Usually, a single cell type will be grown in isolation (e.g. cardiomyocytes, cardiofibroblasts), whereas in the in-vivo situation, different cell types develop together and function as units. Also, in-vitro systems have no blood supply, whereas in-vivo the circulation can contribute to heart disease process by providing a pathway for disease causing molecules between the heart and the rest of the body.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Initial pilot studies have been conducted (under authority of a previous licence) to establish the lowest appropriate group sizes for investigation of cardiac function and hypertrophy in the rat and mouse TAC models. Estimated numbers of rats and mice have been based on these group sizes for the appropriate comparison of treatment and control groups using a randomised block design.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Expert statistical advice has been obtained from our Institute Statistics Department and online tools (e.g. NC3R's Experimental Design Assistant) have been utilised during the experimental design phase of this project. For all our studies we follow ARRIVE guidelines to ensure good laboratory practice and transparent scientific reporting.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The steps in the protocol have been chosen to provide the maximum detailed information necessary to address our research questions whilst at the same time ensuring that the animals under investigation experience the least pain, suffering, distress or lasting harm.

Use of methods that permit serial measurements in the same animal allows for optimisation of the number of animals we plan to use. For example, echocardiography permits a range of cardiac parameters to be determined at a number of different time-points, non-invasively in a single animal. The ability to carry out serial imaging allows the investigation of the evolution of heart disease and the potential influence of dietary, or drug or gene transfer interventions to be carried out in a single group of animals removing the need for multiple groups of animals to be humanely killed at each time point. This also increases the statistical power of the studies whereby the effect of an intervention can be assessed in the same animal over time. Also, since data for each time point come from the same group of animals, there is reduced variability in the data sets and consequently smaller group sizes are required when power calculations are carried out for study design.

Any tissues harvested when the animals are killed at the end of the study, that are not used for the purposes of this specific project, will be processed and stored for use in future studies by ourselves or will be made available to our collaborators.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats and mice will be the only species used in the project.

The TAC procedure is classed as moderate and we will utilise improved (minimally invasive) transverse aortic constriction procedures to induce cardiac hypertrophy, which do not require surgical incision into the chest wall, intubation and mechanical respiration. All surgery will be carried out or supervised by highly skilled and experienced personnel. However, even with experienced hands, there is a risk of surgical complications and death during TAC surgery, especially in mice. We are committed to minimising these as much as possible and are exploring ways to reduce complications by exchanging

experience with other groups and by discussion with the NVS. Analgesics will be administered during all surgical procedures in consultation with the NVS, and for as long as necessary during the recovery phase.

Why can't you use animals that are less sentient?

Mice are the lowest vertebrate group in which the process of chronic pressure overload and hypertrophy in the heart can be fully interrogated. The TAC procedure is limited to adult mice or juvenile and adult rats due to the demanding surgical skill requirements. With current technology, it would not be possible to miniaturise the procedure to use in a more immature life stage. The procedure cannot be conducted in terminally anaesthetised animals because the process of cardiac hypertrophy and cardiac dysfunction develops over a period of weeks.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals that have undergone TAC surgery will be monitored on a daily basis for signs of pain, to check wound appearance and to monitor for clinical signs of heart failure. Any animal giving rise to concern will be scored according to a numerical scoring sheet for pain/discomfort/distress in mice & rats, and if necessary will be humanely culled.

Regular non-invasive monitoring by echocardiography will typically identify detrimental changes in heart function prior to the development of clinical signs.

To reduce stress, animals will be acclimatised for short periods in metabolic cages and will be familiarised with the blood pressure recording procedure prior to the actual measurement phase of the study.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow best practice guidance published by the NC3Rs and the Laboratory Animal Science Association (LASA), as well as adhering to ARRIVE guidelines for appropriate transparent reporting of our research outputs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The Project Licence holder and members of the research team will ensure continued professional development in the 3Rs area through regular attendance at meetings/workshops such the Animals in Science Regulation Unit (ASRU) annual meeting, 3Rs workshops/ symposiums held at local or national Research Institutes, and also attendance at local training workshops organised by the NTCO.

Home Office

NON-TECHNICAL SUMMARY

168.Role of platelets in cardiovascular disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

heart, HIV, pollution, cardiovascular

Animal types	Life stages
Mice	adult, neonate, pregnant, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to understand how substances produced naturally by the body or introduced to the body deliberately as medication or accidentally as pollution may increase or decrease the risk of heart diseases such as a heart attack.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Although much is known about heart disease, the causes are not fully understood and many therapies are not fully effective so that heart disease remains the biggest cause of death and ill-health in much of the world. In addition, because HIV is now treatable, people living with HIV grow old and develop heart disease. There is evidence that they are more susceptible to heart disease and this may be linked to side-effects of drugs used to treat HIV. Our work will also allow us to understand the side effects of drugs used to treat e.g. HIV so that these drugs can be made safer. In addition, we shall understand which components of pollution increase the risk of heart disease so that steps can be taken to reduce human exposure to these pollutants.

What outputs do you think you will see at the end of this project?

We will generate new information in the form of data sets that will be published in scientific journals and presented at scientific conferences that will provide better understanding of the processes of heart disease. We will produce data relating to which pollutants are most harmful to human health and which drugs used in the treatment of HIV are least likely to increase the risk of heart disease.

Who or what will benefit from these outputs, and how?

During the course of this project we shall increase understanding of the causes of heart disease in the general population as well as specific communities. Our work will open up new avenues of research and so will benefit the scientific research community, especially scientists and clinicians with an interest in heart disease and/or HIV. Our work in the field of pollution will benefit organisations such as the British Heart Foundation who are interested in raising public awareness of air pollution and it will be of benefit to vehicle and fuel manufacturers who wish to make their products less toxic. Our work in the HIV field will be of benefit to clinicians treating patients and those involved in setting clinical guidelines as well as patients themselves as we cause therapies to have fewer side-effects.

How will you look to maximise the outputs of this work?

We will continue to communicate our research via social media and through internal and external outreach events. We also publish and communicate our work through a range of scientific networks so that we reach a wide target audience. We also have established contacts in the pharmaceutical industry and HIV patient groups who will be updated on our research. We shall publish all of our data. Where data is not suitable for conventional journals, we shall use open access online platforms. We shall also disseminate our findings via charity networks such as the British Heart Foundation and HIV charities as well as environmental groups. This will in part be achieved by engaging with their social media channels.

Species and numbers of animals expected to be used

- Mice: 2000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using adult mice as we need to work with a mammalian species that can be genetically altered and which mimics the combined effects of the lungs, heart, immune system, liver, blood and blood vessels. We work with adult mice as we need these organs and systems to be mature and we need an animal that is physically large enough to monitor.

Typically, what will be done to an animal used in your project?

Animals that are genetically altered will be bred and then injected with experimental substances usually once but occasionally more often. Some animals may be anaesthetised once and have substances such a pollutants introduced into the lungs via a tube in the airways Animals will undergo procedures involving measurement of blood clot formation under general anaesthesia to determine the effects of experimental substances on this process. Animals will be killed at the end of the procedure without regaining consciousness.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals are expected to feel only brief discomfort due to injection.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severity level is mild for all animals.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animal studies are required because we need to assess the combined effects of substances upon the heart, blood vessels and immune system . This cannot be replicated artificially and so animal studies are required. In addition, we need to deliver substances to blood in a manner that replicates human exposure. This sometimes requires delivery of substances to the lungs of living animals to replicate inhalation and, at other times, the administration of drugs that need to pass through the liver before becoming active.

Which non-animal alternatives did you consider for use in this project?

We have considered analysis of human cells from donated blood and clinical studies using human volunteers. We will use these models in some of our studies but they are not always suitable.

Why were they not suitable?

Studies with human blood do not mimic inhalation and drugs may not be processed to their active form as they are by animals and humans. It is not always possible to administer drugs to human volunteers for ethical reasons and in the field of HIV it is not possible to stop drug treatment in patients as they will become ill as HIV reactivates in the body.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated mice based on the anticipated numbers of experiments, the numbers of experimental groups and the numbers of mice in each group. The estimates are based on similar studies we have conducted in the past using similar protocols.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use the NC3Rs Experimental Design Assistant and consult with the college Statistical Service to design experiments with the fewest animals. We also collect tissues and measure cardiovascular function in the same animals to reduce animal numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Where possible, we induce responses that are entirely reversible. This allows us to assess multiple responses in an individual mouse (e.g. before and after administration of test reagents reduces mouse use by 50%). In some experiments, we are also able to obtain several responses in individual animals which reduces mouse use by greater that 50%.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Animal suffering is minimised by conducting protocols under terminal anaesthesia. This is a refinement of the original methodology which involved death as an endpoint and inflicted considerable pain and suffering. We regularly test for effective anaesthesia during procedures by assessing the toe pinch reflex and administering additional anaesthesia if necessary. There is the potential for suffering when substances are administered to conscious animals or temporally anaesthetised animals prior to terminal procedures. Suffering is minimised by careful aseptic technique and administration of substances by skilled scientists.

Why can’t you use animals that are less sentient?

Most procedures are conducted under terminal anaesthesia. When substances are administered before the main experimental procedures, it is usually less painful to do so in conscious animals rather than giving an anaesthetic from which the animal recovers. We need to work with mice because we need to work with mammals since they have lungs, immune systems and liver function that are similar to humans. We also need to work with adult animals as we need a fully developed cardiovascular system and we need to work with animals that are fully grown from a practical perspective.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

When animals are recovering from anaesthesia they will be monitored continuously for signs of distress and will be kept warm using a recovery box. Animals receiving injections will be monitored daily by the PIL holder. Where problems arise, we shall consult the NACWO and veterinary surgeon and offer pain relief, treatment or cull animals as appropriate.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

LASA guidelines and ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The license holder is very experienced in 3Rs research and regularly attends NC3Rs organised events. He makes efforts to keep up to date on 3Rs best practice and advances.

Home Office

NON-TECHNICAL SUMMARY

169. Small animal models for investigating SARSCoV-2 pathogenesis and for evaluating antiviral compounds

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Coronavirus, SARS-CoV-2, Pathogenesis, Antivirals, Therapy

Animal types	Life stages
Mice	juvenile, adult
Hamsters	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

1. To study SARS-CoV-2-associated pathogenesis in hamster and mouse models.

2. To evaluate the efficacy of known and experimental antiviral drugs and antibodies in these models

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease-2019 (COVID-19). This virus emerged in late 2019 in China and spread rapidly across the world causing a pandemic. The virus has caused over 600,000 deaths so far world-wide. The most important clinical features of COVID-19 are pneumonia with fever, cough and difficulty with breathing. The virus infects cells in the lung and triggers an adverse immune response (cytokine storm). Severe disease can also affect multiple organs of the body. To date there is no vaccine or antivirus treatment available, and they represent a priority area of research for the global control of the pandemic.
The virus can infect human or animal cells in culture, and such infection systems are proving useful in advancing our knowledge on the basic processes related to the molecular biology of the virus. On the other hand, the availability of (and accessibility to) animal models is limited. Animal models that mimic human disease are crucial in studies on the processes and factors that lead to the occurrence of the disease, and in the assessment of the potency of antiviral compounds which is an essential step in a drug development pipeline.
Here, our aim is to establish hamster and mouse models to investigate SARS-CoV-2 pathogenesis and to evaluate in them the efficacy of antiviral compounds that are under development both at our Institution and elsewhere.

What outputs do you think you will see at the end of this project?

Objectives 1 and 2: We hope to gain new insights into the biological mechanisms of virus infection and spread in the host organism. It is not clear how the virus spreads in the host and what cell types are affected by the infection. We also hope to gain enhanced understanding of the basic pathology in tissues, of how viral and host factors modulate host immune and inflammatory responses, and how the complex interplay between them contribute to COVID-19 disease.
Objective 3: There is an urgent need for an effective therapy for COVID-19. Animal models are essential for pre-clinical assessment of promising new therapeutics. The drug/antibody efficacy assessment studies planned herein will allow us to identify lead compounds that can be taken forward for clinical end-use. Our studies will identify potential drug-and antibody-resistant viruses and assess them in our efficacy model. This will inform future treatment options (e.g. use of drug or antibody combinations) that would mitigate the effect of resistance-associated mutations.
The results of our study will be disseminated in the form of publications, presentations at meetings, and through

press releases and other social media platforms.

Who or what will benefit from these outputs, and how?

These research outputs will be published in leading interdisciplinary journals so that they can be used by a variety of research and health professionals. The short-term benefits will primarily be specific to scientific researchers investigating the COVID-19 disease. Medium to longer term beneficiaries will include drug developers and clinical teams who will be able to use these new insights to further develop novel and more rational therapeutic and patient management strategies to reduce disease burden.
The insights gained will eventually be translated to the human context in collaboration with our local clinical colleagues. In addition, through interactions with colleagues in pharmaceutical companies, we will actively seek opportunities to use the knowledge gained to further develop novel therapeutic approaches.

How will you look to maximise the outputs of this work?

We will have extensive collaborations with medicinal chemists, virologists, and pharmaceutical partners. We will disseminate new knowledge in the form of publications, presentations at meetings, and through press releases and other social media platforms.

Species and numbers of animals expected to be used

- Mice: 1000
- Hamsters: 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Small animals such as mouse and hamster have been reported to be suitable for studies with SARSCoV-2. Standard golden Syrian hamsters are susceptible to infection with the virus and present clinical features resembling those found in human patients, including contact transmissibility. While standard laboratory mice are resistant to infection by a natural isolate of SARS-CoV-2, they can be infected with a mouse-adapted mutant virus. Furthermore, mice that express human angiotensin-converting enzyme 2 (hACE2) protein, a host receptor essential for virus entry into target cells, can be infected with a natural isolate of the virus.
The choice of juvenile and adult life stages is based on published reports describing their suitability for this type of study.

Typically, what will be done to an animal used in your project?

Animals will be infected via intranasal route with SARS-CoV-2 virus. In some cases, normal mice will first be given a dose of a harmless adenovirus that express the human ACE2 protein prior to infection with SARS-CoV-2.
In protocols 3 and 4, animals will be administered with drugs or antibodies prior to, and after, infection with SARS-CoV-2.
Animals will be on procedure typically up to 10 days (in some cases this period could be extended up to 30 days).

At the end of all procedures the animals will be euthanised and tissue and organs will be harvested for analysis as described below.

What are the expected impacts and/or adverse effects for the animals during your project?

Most procedures to be carried out are associated with moderate severity rating. Infected animals are expected to experience weight loss, possibly abnormal breathing, and lung abnormalities that could subsequently progress to more severe form (consistent with severe pneumonia found in human with COVID-19 disease). We do not expect any animal to exceed moderate rating. Any animals showing 3 moderate signs or any single severe sign will be humanely killed. Mice undergoing all procedures will be monitored carefully and regularly by the NVS to minimise distress and suffering.
All substances will be administered at doses known to be non-toxic, based on the availability (in the literature or otherwise) of their toxicity profile, and also based on their *in vitro* cytotoxicity profile.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most procedures to be carried out are associated with moderate severity rating. For both hamsters and mice, we estimate overall approximately 50% of animals experiencing Mild severity and 50% Moderate. We do not expect any animal to exceed moderate rating. Any animals showing 3 moderate or any severe signs will be humanely killed.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Only living animals can exhibit the complex interactions between cells in tissues, the immune system and the virus, which together, help determine how the virus can spread in different tissues and organs, and how it causes the disease Therefore, animal models that mimic human disease are crucial in studies designed to understand the disease process and to test compounds for their ability to inhibit virus infection. Those showing strong inhibition can then be considered for treatment.

Which non-animal alternatives did you consider for use in this project?

SARS-CoV-2 can infect human or animal cells (such as the human epithelial cells A549 or CALU-3 or the monkey kidney cells VERO) in culture. We use such cell culture systems to perform the biological analyses of the virus in the context of infection. We also such systems to perform studies to identify and evaluate potential compounds that can be developed for therapy.

Why were they not suitable?

While these cell-based systems are useful in advancing our knowledge on the basic processes related to the

molecular biology of the virus, they do not mimic many aspects of the complex multicellular environment of tissues and organs of living animals. Only living animals exhibit this.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We propose to restrict the number of animals to a minimum required to provide statistically significant analysis. From previous publications and upon expert advice from bio-statisticians, we will use 3 animals per cage and each cage will represent therefore n=1 experimental unit. Each condition (e.g. dose 1 of drug/antibody “x”) will be tested in n=4 experimental units, referred collectively as a “group”. Hence a group is formed by 4 experimental units (12 animals placed in 4 different cages). Each group will be split in two distinct experiments. The condition is therefore tested initially on 2 experimental units (2 cages, 6 animals) and then repeated independently once in another 2 experimental units giving n=4.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Sought advice from bio-statisticians to determine animal group size. Data from our cell culture work also helped inform experimental design – e.g. the type viruses (WT or mutant) and/or the type and doses of drugs or antibodies to be used. Thus, results collected prior cell culture studies mean a reduced number of animals can be used to resolve questions of pathogenesis using information obtained from in vitro studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have factored in sharing of tissues where appropriate with our collaborators to minimise the number of animals in the study.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use hamster and mouse models in our studies, as they have been reported to be suitable for studies with SARS-CoV-2.
Animals will be infected via intranasal route with SARS-CoV-2 virus. In some cases, normal mice will first be given a dose of a harmless adenovirus that express the human ACE2 protein prior to infection with SARS-CoV-

2.
In protocols 3 and 4, animals will be administered with drugs or antibodies prior to, and after, infection with SARS-CoV-2.
Animals will be on procedure typically up to 10 days (in some cases this period could be extended up to 30 days).
At the end of all procedures the animals will be euthanised and tissue and organs will be harvested for analysis as described below.
The proposed protocols are designed to cause least pain and suffering.

Why can't you use animals that are less sentient?

The animals chosen are the least sentient animals that are suitable for our studies.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals will be monitored daily and will be humanely killed if necessary, following veterinary advice. We will use local AWERB and other guidelines for dosing and for blood sampling, establishing clear humane endpoints, monitoring animals frequently, not allowing infections to develop into severe illness.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow Home Office and NC3R guidelines and those published such as:
Carbone and Austin (2016) Pain and Laboratory Animals: Publication Practices for Better Data Reproducibility and Better Animal Welfare. *PLoS ONE* **11(5)**: e0155001. doi:10.1371/journal. pone.0155001.
Percie du Sert *et al.* (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* **18(7)**: e3000410. https://doi.org/10.1371/journal. pbio.3000410.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Keeping abreast with any new information and guidelines through the NC3R website and through our animal facility. We will constantly liaise with the animal facility staff and attend relevant presentations from them to ensure that we are up to date with the information and that they are implemented.

Home Office

NON-TECHNICAL SUMMARY

170. Somatosensory processing

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

somatosensation, spinal cord, brain, nerve

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Rats	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project seeks to find ways to further our understanding of painful conditions in order to improve pain relief by studying how injury alters the way in which sensory information is processed in the nervous system.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Current pain relief options are only partially effective or patients may not respond at all to treatment and many of the available medications have adverse side effects and/or abuse potential. This poorly managed pain is extremely debilitating and significantly reduces patient quality of life. To develop urgently needed novel therapies we need to better understand the aberrant processing of sensory signals within peripheral nerves, spinal cord and brain. This need is well illustrated by the neuropathic pain that results from damage to nerves by chemotherapy treatment in cancer patients. These conditions can be so debilitating and poorly managed by current therapies that they can result in cessation of life-prolonging chemotherapy treatment.

What outputs do you think you will see at the end of this project?

Sex influences our sensitivity to pain and how we respond to injury. Women are more sensitive to pain and are more likely to develop persistent pain following injury, experiencing more severe symptoms. There is gradually accumulating evidence for sex differences in underlying pain neurobiology but this is poorly understood. Therefore, the proposed research which aims to further our understanding of pain neurobiology, in both sexes, should provide new information that will be required in the longer term to develop and improve pain management for both sexes. It is anticipated that this work will yield exciting data, that will have a significant impact in the field and will be published in high quality Neuroscience or Physiology journals.

Who or what will benefit from these outputs, and how?

Less than 50% of chronic pain patients achieve pain relief but this is often only partial and is accompanied by adverse side effects. For these individuals pain literally dominates their daily existence, drastically reduces their quality of life and markedly impairs their capacity to be independent of family and friends, contribute to the workforce and be an active functioning member of society. Therefore, the proposed research that should provide new information should have a broad impact: i) the scientific community – pain researchers; neuroscientists; ii) health professionals and ultimately their patients iii) the Pharmaceutical Industry iv) Government and policy makers and v) the wider community.

How will you look to maximise the outputs of this work?

Collaboration: I have previously collaborated on translational pain research projects to produce high impact publications that have led to significant publicity (online and print newspapers, medical forums and on television) and current funding applications for an early phase clinical trial. I will similarly exploit findings from the projects proposed over the course of this project licence.

Dissemination of knowledge:

The scientific community – pain researchers; neuroscientists: Publication of results, including negative findings, in high quality Neuroscience or Physiology journals ensuring free Open Access. I will present and organise workshops at national and international meetings.

Health professionals and ultimately their patients: In addition to presentation at national and international meetings where there is significant healthcare professional participation, I will also further this through my active membership of a National Pain and Research Community one of whose founding objectives is to disseminate new, relevant research to clinicians and patients through its annual meeting and community website. The overarching aim of this grouping is to ensure that clinical practice in managing chronic pain is informed by research and that current research in chronic pain is relevant to clinical practice.

The Pharmaceutical Industry: Intellectual property management, licensing and technology and knowledge transfer is supported by a dedicated team within my Institution. It has extensive expertise in the protection of intellectual property arising from research. Regular meetings will be scheduled to discuss developments on this research programme and to ensure that potential outputs are maximised.

Government and policy makers.

I will also continue to the National Pain Research Community, as one of its key functions is to ‘disseminate relevant research findings to health service policy makers, highlighting relevance to future and current policy’. To ensure a direct impact, I have been a member of a Service Improvement Group that reports back to a Government National Chronic Pain Steering Group whose remit is to ensure that clinical practice in managing chronic pain benefits from current pain research that is clinically relevant.

v) The wider community.

The Institution’s Press Office has a strong record of communicating research to the public (both directly and through the media). They have promoted prior work from my lab. It received significant press interest and was covered widely in online and print newspapers, medical forums and on television. Furthermore, I have previously and will continue to contribute to outreach activities such as hosting school pupil laboratory visits.

Species and numbers of animals expected to be used

- Mice: 6000
- Rats: 2500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We study mice and rats because their nervous system and development are very similar to that of humans. Moreover, scientists have created many genetically altered mouse and rat lines that allow us to dissect in fine detail how the nervous system processes sensory information. This will help us to identify how this changes, and leads to debilitating conditions, following tissue injury or nerve damage. We study neonatal, juvenile and adult stages because we wish to understand how this system develops and how it may be altered by injury at these different life stages.

Typically, what will be done to an animal used in your project?

Typically an animal will receive a local tissue injury or injection of a chemotherapy agent which results in nerve damage. To study the changes that this tissue/nerve injury induces within the nervous system, following cessation of the animal - skin, peripheral nerve, spinal and brain tissue will be isolated for anatomical or functional analysis. In some studies, the symptoms that these injuries produce such as hypersensitivity to touch or cold will be assessed by monitoring the animals' behaviour and the ability of pharmacological agents to reduce these symptoms will be assessed. Some studies will be carried out in genetically modified animals to enable in depth analysis of the changes within the nervous system that cause hypersensitivity symptoms that is needed to identify new therapeutic targets.

What are the expected impacts and/or adverse effects for the animals during your project?

Tissue injury will be conducted under general anaesthesia. Following tissue injury and chemotherapy–induced nerve injury animals become hypersensitive to stimuli such as touch and cold. However, these animals feed and drink normally, put on weight normally and do not modify their general behaviour. The duration of these symptoms depend on the type of injury and can range from hours to weeks. However, in all injury models there will be a defined period when symptoms peak and in most studies we will limit investigation to the shortest period feasible. Notably any genetically modified animals used do not have adverse impacts.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Just under a half of animals will be used for establishment, maintenance and production of genetically altered animals of which 1/4 will simply be used for harvesting tissues and will fall will into a subthreshold or mild limit of severity. Generally, most experimental studies will be conducted on mice (~3/4) with a smaller proportion carried out on rats (~1/4). Most experimental animals will be used to study tissue injury with 2/3 experiencing tissue injury (moderate severity) and 1/3 acting as control comparisons (mild severity). A small proportion of experimental animals will be used to study chemotherapy-induced neuropathy with ½ receiving chemotherapy (moderate severity) and ½ receiving a control treatment (mild severity). Animals killed under terminal anaesthesia for extraction of blood but not experiencing any other procedure while alive will be classed as non-recovery but will be relatively few in number.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this work is to further understanding of pain conditions by working out how injury alters the processing of sensory information by peripheral nerves, spinal cord and brain. We aim to identify the connections within this system that malfunction in these conditions with the aim of improving the development of pain relieving medications. We therefore need to conduct these studies in a species that develops these symptoms and has a comparable sensory nervous system with similar complexity.

Which non-animal alternatives did you consider for use in this project?

In vitro cell culture or theoretical modelling

Why were they not suitable?

The normal functioning and wiring of this system is not as yet fully understood and so these pathways cannot be modelled in vitro or theoretically, so there is no feasible alternative that would entirely replace the use of a living animal

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals has been estimated based on experience gained under my previous Home Office license. In this regard, the work plan and nature of experiments covered by this project are similar in design to those covered under my previous license. Hence, I have based estimates on successfully obtaining funding for x3 3-yr post-doctoral positions and X3 3-4yr PhD studentships and completion of x2 current studentships to execute the work detailed in this license, and the average yearly animal use per group member returned under my current license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The following points specifically address experimental design that reduces animal numbers. In functional studies, up to 4 preparations can be obtained from any one animal, greatly increasing successful data acquisition from an individual animal and thereby reducing the total number of animals required. In proposed functional studies, recordings will be made from neurons processing specific symptoms using genetically modified animals. In this way, data will be directly obtained from the key neuronal populations involved. This reduces usage as the alternative approach of recording data from random neurons would vastly increase the numbers of animals required to obtain relevant information.

All studies will be conducted in both sexes and analysed by sex. Although somewhat counter-intuitive studying these basic questions in both sexes will lead to a reduction in the numbers of animals used. From our own experience, pooling data from both sexes can lead to a diluting of effect size or worse a misinterpretation of findings that can lead to additional experimentation that may be eliminated by addressing these questions independently in both sexes at the outset.

Importantly, the numbers of animals required for a given study is worked out using statistical analysis of pre-existing data or if this is not possible in preliminary data collected from a very small group of animals to ensure that subsequently only the minimum numbers that are required for the study are used. Our experiments are also designed to reduce the number of variables to as few as possible and thereby reduce the number of control groups required. In this respect, we will always consider carefully whether it is important to include ‘naïve’ as well as ‘vehicle’ control groups in experiments, or if the latter alone is sufficient for interpretation of results. In some cases, the uninjured side can be used as a control.

To ensure best practice in statistical analysis and experimental design all new staff members working under this license will attend the in-house ‘Experimental Design Course’. Planned experiments are discussed regularly within group meetings to ensure all are correctly controlled and to facilitate sharing of tissues/data for the most effective use of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

My group routinely perform pilot experiments or use pre-existing data from the lab where possible to determine sample sizes required by power calculations. We standardly base power calculations on the ability to detect at least a 20% difference using online tools. We also seek advice when required from a

local experimental design expert and NC3Rs Experimental Design Assistant as appropriate. Planned experiments are discussed regularly within group meetings to ensure all are correctly controlled and to facilitate sharing of tissues/data for the most effective use of animals.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats and mice are chosen for this work given that much of the current understanding of this topic has been carried out in these species, knowledge that is an essential foundation for the studies proposed here. Moreover, the mechanisms and neural connections established in these species are comparable, to the extent that this can be addressed, to that in humans, underscoring the relevance of this work for humans. The models to be employed should not modify general behaviour (grooming, eating, sleeping, social interactions) although a localised tenderness of one limb may be present which is required in order to investigate biological mechanisms in order to guide future pain relief strategies. In all studies we will seek to limit the timeframe of the tenderness and the majority of studies will be conducted on isolated tissue preparations.

Why can’t you use animals that are less sentient?

A mammalian model is required to study a nervous system with a high degree of functional, anatomical and developmental similarity and complexity. This is required to generate relevant findings on which to base future therapeutic strategies. The duration of the pathophysiological processes to be studied prevent using mice or rats under terminal anaesthetic.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In line with institution policy, we will adopt the latest techniques in animal handling (e.g. cupping) to significantly reduce the stress associated with procedures. For behavioural testing, all animals are well habituated to the test scenario and returned as quickly as possible to their home cage.

Furthermore, where possible, the least invasive methods for dosing and sampling will be applied. Anaesthesia and analgesia will be provided where suitable (e.g. for humane restraint, during and recovery from surgery). To reduce infection risk, the best aseptic technique will be used during surgery (e.g. sterilization of instruments between animals, full surgical drapes). When possible early endpoints will be used in the models.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Our institute employs a dedicated team of veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consults closely with this team and takes full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures. We have also consulted the NC3Rs research strategy paper by Prescott MJ, Lidster K (2017).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our institute employs a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consult closely with this team and take full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures that are continually being updated. Our university is also in the process of adopting the improved rodent handling methods that reduce animal stress (detailed by Hurst et al. Nat Methods 2010) and our animal facilities now provide environment enrichment as standard. My group will adopt these methods alongside the staff in our animal facilities. We will also take full advantage of the annual 3R’s seminar day organized by the University’s Animal Welfare Committee to find out about pioneering developments in best practice.

Home Office

NON-TECHNICAL SUMMARY

171. State-dependent neural information processing and brain function in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b) **Key words**

brain, neuron, sleep, ageing, hearing

Animal types	Life stages
Mice	adult, pregnant, juvenile, neonate, aged, embryo
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to better understand how the brain processes information at the level of neural circuits and how it changes in brain disorders or ageing. In addition, this project aims to develop novel technologies to realise them.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our brain is one of the most important organs in our body. Abnormalities in the brain cause brain disorders that often lead to life-long disability.
This work will investigate how internal states of the brain are regulated and how internal states of the brain affect various brain functions in health and disease.
An internal state of the brain, called "brain state", can be defined by ongoing electrical activity in the brain. While the brain is never at rest, brain states dynamically change over multiple timescales. A typical example is the sleep-wake cycle where brain states changes in a range of seconds to hours. Our sensory percepts can be also changed depending on those brain states. For example, our hearing ability can vary across the sleep-wake cycle. Moreover, ageing is also associated with changes in brain states. For example, brain activity typically becomes slower in the aged brain. This is particularly acute in hearing: even when hearing threshold is held, the ability to comprehend speech sounds in noisy environment decreases as age.
A better understanding of the neural basis of brain states is a fundamental issue in modern neuroscience.
In addition to brain states in health, abnormalities in brain activity are associated with brain disorders including neurodegenerative diseases (such as Alzheimer's disease) and neurodevelopmental disorders (such as autism).
A better understanding of brain states in disease can make a huge impact on our society.
For example, in 2010 alone, there were approximately 45 million cases of brain disorders in the UK. The economic cost of brain disorders was over £120 billion. For example, Alzheimer's disease (AD) is the most common cause of dementia, which affects around 850 thousand people in the UK, with a cost of £13.9 billion.
Despite this enormous societal and economic burden, our knowledge about how brain disorders are caused and even how the healthy brain works is still limited. Because of this, no effective treatments are available for most brain disorders.
To tackle this challenge, not just clinical research, but also pre-clinical animal research is essential. Because the brain consists of complex networks of neurons and non-neuronal cells, it is important to gain an access of individual cells in living animals by taking and developing advanced technologies.
Thus, this work will help (1) understand principles of how neuronal circuits operate in the healthy brain and (2)

gain insights into the mechanisms of brain disorders and the ageing process. In addition, because existing tools to investigate brain functions have their limited ability to monitor and control brain activity in vivo, this work will also help develop and validate novel devices to monitor and control brain activity in an unprecedented manner.

What outputs do you think you will see at the end of this project?

The main outputs will be

- Conference presentations
- Publications in peer-reviewed journals
- Publicly available data files for reproducibility and reduction
- Novel neural implants and interfaces, some of which will become commercial products

Who or what will benefit from these outputs, and how?

Because this study is still basic science, primarily beneficiaries from the output of this study will be academia. As we will publish our findings, this impact will be realised.
Because we believe that a better understanding of brain state is a fundamental issue, long-term (>10 years) impacts can be vast, from advancing human knowledge to clinical applications for brain disorders, such as Alzheimer's disease, and the development of novel artificial intelligence to help human activities in multiple domains.

How will you look to maximise the outputs of this work?

Wherever possible, we will disseminate the project by presenting it at national and international conferences and by publishing a paper with an open-access option.
Along with publications, we will make data publicly available to facilitate reproducibility and reduction.

Species and numbers of animals expected to be used

- Mice: 9700
- Rats: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

There is no feasible alternative that would entirely replace the use of these types of animals at the life stages in order to investigate state-dependent brain functions in health and disease. Constitutive GAAs are necessary to model genetic defects of human disorders/diseases, such as Alzheimer's disease. Conditional GAAs are necessary to modify gene expression in a spatially and temporally restricted fashion. Aged animals are necessary to better understand age-related changes in neural information processing at the level of neural circuits.

Typically, what will be done to an animal used in your project?

A large proportion of animals will be used for breeding. Some animals will undergo surgical procedures under general anaesthesia in order to prepare them for physiological and behavioural assessments later. The surgical procedures will allow placement of recording and stimulation devices on the skull and head/neck muscles. After a 1-week recovery period, the animals will be habituated with an experimental environment. Then behavioural and physiological assessments will be carried out. After completing all procedures, the animals will be killed by a schedule 1 or non-schedule 1 procedures. A typical duration of procedures will be between two and six months. A small number of animals will also undergo procedures during their development as a model of neurodevelopmental disorders. The animals will be also examined for their behavioural and physiological characteristics once they mature. Another group of animals may be allowed to reach older ages (up to 2 years) in order to study age-related changes in brain activity. The procedures are similar to those for young animals while a longer recovery period will be allowed.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals undergoing surgical procedures may suffer from post-surgical pain/discomfort for several days. A few animals may also lose moderate amounts of weight compared to control animals during longer procedures that also involve steps such as water-restriction for behavioural training, even though they will receive a limited amount of water and an unlimited amount of food every day.
Regarding aged animals, all C57BL/6 mice will develop age-related hearing loss (presbycusis). Although this strain has a low susceptibility to tumours, this strain shows a susceptibility to diet-induced obesity and hair loss associated with overgrooming.
CBA or CBA/Ca mice were originally developed for longevity. However, it is known that ageing CBA mice can develop spontaneous hepatomas. Male CBA/Ca strain develops a mild adult onset diabetes obesity. Because most GAA mice used under this protocol will have a genetic background of either strain and because only “knock-in” animals will be used for this purpose (i.e., ageing), the effect of ageing will be similar to the one expected for the background strain and hence well-established. Nevertheless, other age-related conditions may appear

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Expected severities can be categorised as either Mild or Moderate.
Mild severity is expected for a very small number of animals that are subject to an unconventional genotyping method (e.g., blood sampling), rather than ear notching.
Moderate severity is expected for animals that are subject to surgical procedures, pharmacological or non-pharmacological interventions, and ageing.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The quantitative assessment of sensory-evoked neural activity and behaviours (including sleep states) requires in vivo studies. There is no alternative that would entirely replace the use of a living animal that would allow the objectives to be met.
In some of behavioural experiments (such as classical conditioning), aversive stimuli (such as air puff) may be more suitable than positive reinforcement in order to reduce their training periods and the number of animals used.

Measuring neural responses in conscious animals is necessary to investigate the neural mechanisms of sensory perception and other brain functions because neural activity in conscious animals significantly differs from that in unconscious ones.
Monitoring electrical activities of the brain (EEG) and muscle (EMG) is commonly used to objectively assess brain/behavioural states because of its less invasive nature. EMG measuring is essential to distinguish wakefulness and rapid-eye-movement (REM) sleep states. Currently there is no alternative approach to the best of my knowledge.

Which non-animal alternatives did you consider for use in this project?

The following alternatives were considered:

- human subjects in
- vitro systems

Why were they not suitable?

It is not ethical to conduct experiments on human subjects, especially because this project requires invasive procedures.
In vitro systems do not allow us to assess sensory-evoked responses and does not produce behavioural outputs.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals have been estimated based on:

- statistics from pilot experiments
- statistics from my own published works and similar works experience from
- the previous PPL

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I took the following steps:

- Designed each study to determine control groups and experimental units (e.g., a single animal, a single cell)
- Considered outcome measures to achieve the goal of the study together with experimental procedures
- Considered a range of inclusion and exclusion criteria
  Considered whether randomisation and/or blinding can be taken
- Estimated sample size
  Considered potential statistical methods to assess the results
-
-

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

I will use the following measures where possible:

- efficient and well-planned breeding
  performing a pilot study before starting a large-scale study to ensure the estimate of sample size and
- no toxicity adopting advanced data analytical approaches to maximise the outcome sharing non-used
- tissue with other scientists and storing brain samples for future studies
-

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use rats and mice. Rats are suitable for experiments that require to accommodate a certain methodology/technology, whereas mice are more suitable to utilise genetic technologies and some behavioural experiments. To complement both advantages, we will use both species depending on the objective: when any experiments require genetic approaches, we will use only mice. However, rats will be used when certain experiments (such as validating a novel device) cannot be applied to mice. GAAs are required to express exogenous genes to monitor and control brain activity at refined spatio-temporal resolution or to model brain

disorders, such as Alzheimer's disease to develop novel pharmacological or non-pharmacological intervention strategies.

Why can’t you use animals that are less sentient?

To investigate state-dependent brain functions, such as the sleep-wake cycle, non-mammalian species exhibit different patterns of state changes from mammals. Out of mammals, rodents may be recognised as less sentient species. Given available and proven methodologies and technologies, rodents are believed to be most appropriate for this project. In non-recovery conditions, less frequent state changes are expected and brain states differ from those in un-anaesthetised conditions. Thus, experiments in un-anaesthetised conditions are required. Patterns of state shifts (e.g., sleep duration) also change as a function of age. Thus, animals at a more immature life stage will provide a very limited aspect of state dependent brain functions.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All animals in recovery experiments may experience some post-operative pain or discomfort because of surgery. We will use post-operative analgesia to reduce surgical post-operative discomfort. Health condition including body weight, spontaneous movement, head/face swelling, and appearance will be monitored and scored in first several days after surgery.
All animals involved in behavioural assessment under head restraint conditions may experience some discomfort and stress. To minimise stress, animals will be trained to willingly accept the restraint by positive reinforcement and the duration of training will be gradually extended from several minutes over weeks. During training animals will be video-monitored online. If animals show discomfort, experiments will be immediately terminated. Procedures involved implanting a device will be terminated in 6 months. If animals show any deviation from normal health (e.g., weight loss), behavioural training will be suspended to allow them to recover or they will be killed humanely.
To reduce the mortality rate of aged animals during and after surgery, the amount of anaesthesia will be reduced and the duration of surgery will be minimised. Depending on the animal’s condition, we will take a longer recovery period by monitoring health conditions on a daily basis.
Although some of age-related changes (e.g., age-related hearing loss) can be observed only from a certain age, an option to use younger animals will be sought to minimise age-related adverse effects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the guidelines on the NC3Rs website (https://nc3rs.org.uk/3rs-advice-project-licenceapplicants-refinement).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly check the websites of ARRIVE (https://arriveguidelines.org/) and the NC3Rs (https://www.nc3rs.org.uk/)

Home Office

NON-TECHNICAL SUMMARY

172. Studies on vascular diseases and repair mechanisms

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Cardiovascular, AMPK, Breeding, Wire Injury, Blood Pressure

Animal types	Life stages
Mice	adult, pregnant, embryo, neonate, juvenile, aged
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project seeks to understand more about how some very serious and common cardiovascular diseases affect the structure and function of our blood vessels and how drugs or other interventions may improve the health of the vascular system.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cardiovascular diseases, or diseases which indirectly affect the cardiovascular system have a huge impact on our health, cause many people to die prematurely and have a high cost to the National Health Service. The project will increase our understanding of how blood vessels change when subjected to adverse situations such as hypoxia (a low level of oxygen), high fat in the blood or mechanical injury and how this causes changes in their normal function. This will include studying the natural processes which occur over time. This can sometimes help to identify how and why the function changes and maybe identify ways in which new drugs can be developed to target these processes.

What outputs do you think you will see at the end of this project?

We will generate significant new data on blood vessel regulation and how this changes in disease and when vessels repair after injury. We intend to publish this data to share our findings and help advance the field. We may use our animal models to study the effect of novel drugs- for example drugs which inhibit the enzyme AMPK or SK. We may also study the effect of novel ways of delivering drugs to an injured blood vessel to study if this improves the action of the drug or how effective it is in improving the outcome of the treatment.

Who or what will benefit from these outputs, and how?

In the long-term, human health will benefit as data from this project can inform future directions of research in humans using novel drugs or novel surgical techniques with improved results. This may be achievable if we understand more about blood vessel function and repair after injury.
As basic scientists we will benefit by learning more about blood vessel function and, more importantly, how they stop functioning properly when they become diseased. We will find out more about how the fat which surrounds our blood vessels can change during diseases such that it becomes damaging rather than protective. We will also learn more about how blood vessels repair themselves after injury and the effect low levels of oxygen (called hypoxia) can have on a family of lipids which can affect the function of blood vessels.
Students (PhD and Masters) working on this project will benefit by being trained in good experimental design and *in vivo* techniques. Publication of the work we have performed and dissemination in the wider scientific community will more the field forwards.
In the mid- and longer-term these outputs may assist in identification of drug targets which the pharmaceutical industry can use to develop new or improved drugs to treat human disease. Such improved therapies will have a

beneficial impact on human health in the long-term.

How will you look to maximise the outputs of this work?

We aim to publish our research in scientific journals so that our findings can be shared with other researchers working in this area. We also aim to present our findings at national and international meetings so that ideas and novel techniques can be shared and the best approach to investigating our area of research adopted. We collaborate with others in the area so that new skills can be learnt and supporting skills can be used to optimise the programme of research and maximise the outcomes. **Species and numbers of animals expected to be used**

- Mice: 5700
- Rats: 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

For the types of experiments we are performing we need to use adult animals as their cardiovascular systems are fully developed. For experiments where we cause damage to the vessel or to the endothelium (the cells which line the inside of blood vessels), the vessel has to be of a sufficient size to allow access with the fine wire we use for the procedure.
We choose to use mostly mice as this allows us to breed and use mice which are genetically modified (GM), this allows the genome of the animal to be manipulated so that the protein product of a particular gene is not produced (or in some cases the manipulation can be so that more of the protein is produced). This allows us to use animals which are deficient in proteins we are interested in studying- such as an enzyme known as AMPK.

Typically, what will be done to an animal used in your project?

The vast majority will simply be used for breeding, their genotype being determined by a small sample removed from their earlobe at the time of identification.
Some animals will be used to measure blood pressure non-invasively and they will be treated acutely or chronically with experimental compounds given either orally or by injection, up to twice daily for up to one month (depending on route) before they are killed humanely. In some cases, their diet may be modified so that they are fed a high-fat diet for up to 30 weeks- this does not have any overt adverse effects on the mice.
A small subset of mice (or rats) may undergo a surgical procedure under general anaesthetic that will result in damage to the wall of a blood vessel and in some cases and implantable stent will be delivered, after which they are allowed to recover. These animals may receive therapeutic doses of novel drugs that help remodel the induced vessel defect for up to one month after induction of the lesion. This is to study the effects of vascular injury on the structure and function of blood vessels and how they heal after an injury.

What are the expected impacts and/or adverse effects for the animals during your project?

Breeding of mice and modifying their diet should not cause any ill-effects.

Administering drugs to animals can sometimes have unpredictable effects but we try to minimise this by testing the drug in experiments which do not use live animals and we try to use doses which have been used by other researchers or from the manufacturer of the drug where this information is available. When we study pharmacological agents *in vivo* we commence with small pilot studies to minimise numbers of animals exposed initially to establish a safe dose.
Injuring animal blood vessels and then recovering the animals can sometimes produce some adverse effects such as:
An animal not recovering from the anaesthetic- perhaps 1 animal in 100 (1%)
The animal dying during the procedure- this can often be because the blood vessel which is being injured breaks or bursts. This may happen in up to 10% of animals but the animal never regains consciousness.
After the surgery sometimes the stitches or wound can become infected or open up again if the stitches fail (maybe 2% of animals)
Pain – we expect all animals may experience some discomfort during recovery but we minimise this by using pain-killing drugs given before the surgery starts and after it, and also drugs which reduce inflammation.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Protocol 1 of this licence is mild (100% of mice will experience this due to ear notching for genotype analysis, while protocols 2 and 3 are moderate. In protocol 2 some animals may experience discomfort or adverse effects due to repeated injection of pharmacological agents. In protocol 3 we estimate that 100% of animals will experience some post-operative discomfort.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

A blood vessel is a complex tube consisting of multiple layers of different cell types and in a living creature, has a constant, pulsatile flow of blood through it. How these cells interact when the blood vessel is injured or when the amount of oxygen is reduced is crucial to our understanding of vascular diseases. The only real way to understand this in detail is to examine the changes in a live animal, comparing an injured blood vessel to a non-injured vessel and how the structure and function changes over time. Although alternatives can give us some information, they often fail to mimic the complex environment in a live animal, with blood, immune cells and circulating hormones and mediators.

Which non-animal alternatives did you consider for use in this project?

We derive much of our preliminary data from experiments which do not use live animals (for example the use of cells grown in culture) and this helps us to decide on doses of drugs and other conditions for studies which do

use animals. It also helps to cut down the risks of unforeseen problems when we do use live animals. For example, cultured cells can be purchased commercially and may be either human or animal cells. We also maintain other relevant cell lines such as fat cells called 3T3 adipocytes, which we use extensively. From these experiments we can derive a huge amount of data which reduces animal numbers and informs the experimental conditions when we do need to use animals. We also make extensive use of historical samples of preserved tissue and tissue fluids/blood which originally came from animals but can be used in multiple experiments.

Why were they not suitable?

They are suitable for certain experiments which form the bulk of our research. However, in certain experiments- such as measuring blood pressure, blood vessel healing responses or testing the effect of a drug on the cardiovascular system, we need to use a live animal. A live animal has many features which cannot be replicated using cultured cells or tissues removed from the animal for *in vitro* experiments. For example, blood pressure can only be measured in a live animal and the healing response of a blood vessel after injury cannot be studied *in vitro* because other cells and systems which are only active in a live animal (for example blood cells and immune cells, circulating hormones and growth factors) will be involved.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers are based on past experience of running breeding colonies of mice which are used to produce animals for experimental projects. In the proposed research over the 5 years of this licence, the vast majority of these mice will be used for breeding and not for any other licenced procedures. Rats are used on occasion for some experiments where they represent the best means of obtaining reliable scientific data- for example where mice are too small and consequently the size of the blood vessels is too small.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where licenced procedures other than breeding are carried out, experiments are designed carefully in line with best practice to minimise numbers but still obtain statistically valid data. This is normally via tests which use the expected difference between experimental groups and the chances or probability of finding a significant difference- this can inform us of how many animals are required in each group.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Our breeding programme is tightly controlled so that the colony size is monitored and is of a sufficient size to produce the number of animals needed without significant excess. When a member of my research group uses an animal, tissues are shared between multiple users and across research groups so that maximum use is made of that animal.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project involves breeding mice with specific genetic modifications which are of interest to us. Such modification- removing the ability of the animal's genome to produce a specific protein or enzyme allows us to investigate what effect that has on the animal in terms of its cardiovascular system and how its blood vessels function. When required for use these mice are usually killed via a humane method and their tissue and cardiovascular system investigated. On occasion we use anaesthetised animals to study the effects of injecting drugs and the effects of injuring their blood vessels and then studying how the vessel repairs itself over a period of time. In this case, pain, suffering and distress are minimised by good handling techniques, experienced and trained people performing the surgery, use of analgesic drugs and good post-operative care; following all current guidelines.

Why can’t you use animals that are less sentient?

We are investigating the cardiovascular system and so the best models of cardiovascular disease are in mammals and this is why we use mice and rats. It is important that our basic scientific research has what is called "transferability"- where the results have relevance to the disease or condition in humans. This is difficult to argue when using some less sentient species in the type of research we do.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The experimental models we use are accepted by the scientific community and we have published a great deal of peer-reviewed data reported our findings with them over the last 10-12 years. Through this period we have refined our techniques to make each of the proposed procedures have a high success rate.
In all experiments, suffering to the animals is minimised by careful experimental technique and judicious use of analgesics and anti-inflammatory agents at dosages recommended by the veterinary surgeon. Close supervision of the animal during recovery also ensures that any complications can quickly be identified and dealt with to minimise suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We design and report all our studies according to the ARRIVE guidelines which are widely accepted and endorsed by the scientific community: https://www.nc3rs.org.uk/arrive-guidelines

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

There are several sources of information available including the NC3Rs website: https://www.nc3rs.org.uk/
I also regularly correspond with the NACWOs (animal house staff) regarding the best way to maintain our colony of breeding mice and consult with the NVS where any problems arise and to get the most up-to-date information on best use of anaesthetic agents, analgesics and post-operative care of animals under procedure. My employer is committed to a culture of care and respect for research animals and organises an annual 3Rs day.

Home Office

173. Studying macrophage plasticity in inflammatory disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research **Key**

words

inflammatory disease, macrophages, cell therapy

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant
Rats	neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how cells of the immune system called macrophages cause damage during inflammatory disease. Macrophages are versatile cells and depending on the tissue, they can have beneficiary or harmful effects. Through an ambitious and vast research programme, we ultimately aim to identify drugs that can modulate their activity and treat inflammatory disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is pivotal to undertake this research because the animal models we have are reproducible and mimic perfectly the human disease. For instance, we can model the different disease states (i.e. early inflammatory state and late scarring phase) and understand the transition from one to the other. This is crucial in designing small molecules that can be potentially be effective in a specific disease-context.

There is a gap in understanding and targeting inflammatory disease and current therapies involve drugs that silence the entire immune system (immuno-suppressors). These 'non-specific' drugs cause also harm to the human body as they damage the immune system that we rely on to fight against microbes and viruses. This application aims to find more specific drugs that will target one type of immune cell (i.e. the macrophage) while keeping the immune system intact. This strategy can be applied in many unmet clinical needs such as treatment of chronic conditions (chronic kidney disease). The studies we propose will aim to change the activity of the macrophage and 're-program' such that it could help tissue regeneration and repair. These approaches are still challenging to undertake in humans. Although our understanding of human inflammatory conditions are growing every day, any cell therapy aiming to re-program immune cells require the use of animal models. Animal models of inflammatory conditions help us in (i) understanding the disease progression in a controlled environment (ii) targeting specific cell types that worsen the inflammatory reaction.

What outputs do you think you will see at the end of this project?

The project will increase knowledge in understanding and treating human inflammatory disease. The information gained from the project will be translated into human disease where applicable. As we have shown in our previous project, it is highly likely that the new findings will be peer-reviewed and published, contributing to the current scientific knowledge in complex diseases such as chronic kidney disease and skin inflammation. Some findings may also lead to new products that could have potential industrial value.

The ability to understand the role of macrophages in human inflammatory disease is important. It can indeed help designing better treatments. Because these cells change their activity during the inflammatory process (e.g. inflammatory at early stages and 'repair' like at later stages), one can take advantage from this temporal changes to target kidney inflammation. Practically, instead of the convential anti-inflammatory treatments, we can now think of treatments that will support repair - a novel line of research that this project would like to implement.

Who or what will benefit from these outputs, and how?

The short-term beneficiaries will be academic partners and the undergraduate and postgraduate education . The impact of our outputs will also be communicated, where appropriate, as part of lay reports in Press Releases. Other outputs' impact may be realised in the long term. For instance, the translation of the findings through innovation procedures or patent applications is likely to be prolonged in the long-term.

The potential medium term non-academic beneficiaries also include pharmaceutical companies and longer term patient benefits. For instance, we are working with a small pharma company to develop potential drugs that can have an effect on transforming macrophages into 'repair-type' cells. We are excited about these outputs, which can have a clear patient benefit in the long-term.

How will you look to maximise the outputs of this work?

We actively collaborate with researchers around the world in order the optimally disseminate new knowledge. Publication but also press release and/or social media releases are part of the different ways to disseminate research findings. The recently established public databases encourage scientists to disseminate their results in a timely way (e.g. BioRxiv). Furthermore, the increased number of journals publishing negative results show that the scientific community has made knowledge dissemination as one of its primary goals.

Species and numbers of animals expected to be used

- Mice: 700
- Rats: 800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Studying the immune system in rats and mice is an important step in understanding and treating inflammatory conditions. Inflammatory conditions comprise a broad range of diseases where current therapies lack specificity. For instance, when the filtering unit of the kidney (i.e. the glomerulus) becomes chronically inflamed and there is a risk of kidney failure, current therapies are mainly based on corticosteroids, which are non-specific anti-inflammatory agents that affect many tissues within the body causing adverse effects such as weight gain, bone thinning, diabetes and high blood pressure. There is a scientific need to understand how the inflammation is caused (what are the different steps, mediators and their relative importance) in organs/tissues such as the kidney and the skin. By using mostly adult mice and rats, we aim to understand how inflammation occurs and how we can treat it. Mice and rats' immune system resemble closely the one described in humans. For instance, the steps of wound closure in mice and rats' skin is very similar to those observed in humans. Furthermore, the rat model of kidney inflammation in adult animals mimics perfectly the human disease - an important consideration for using these types of animals for biomedical research.

Typically, what will be done to an animal used in your project?

Typically, we will induce kidney or skin or more general inflammation in rats and mice. The procedures described in the protocol 2 are designed to identify the immune mechanisms involved in initiating and/or resolution of the tissue damage and to explore therapeutic targets. A typical protocol will involve the administration of a treatment (e.g. a drug with protective effects) followed by the induction of a brief and transient inflammatory reaction. We are only interested in the biology of early inflammation. We will therefore study the immune cells involved in earl inflammation and seek for protective effects against inflammation. The duration of these experiments will not exceed a period of 7 days following the induction of the inflammatory reaction.

The kidney inflammation (Protocol 3) requires an injection while skin wounds (Protocol 4) will be induced after surgery. The duration of both experiments will be typically between 10-14 days after causing the initial injury. The number of procedures will be kept to minimum. Typically, this will involve a treatment (e.g. a drug that will reduce inflammation).

Both techniques are extremely well-established in our laboratory where our proven track-record identified multiple ways of treating inflammation by targeting the immune system. This work will help in the development of therapeutic approaches to human kidney and skin inflammation.

A typical kidney inflammation protocol will be carried out as follows and will last 10 days after inducing the initial injury

- Injection of nephrotoxic serum (NTS) to induce an inflammatory response and progressive renal scarring.
- Collection of samples (metabolic cages which separates faeces and urine into tubes and blood sampling).
- Tissue harvesting (Schedule 1).

A typical wound healing protocol will be carried out as follows and will last 14 days after inducing the initial injury

- Induction of a skin wound to induce the healing
- Testing a treatment that generally aims to increase the healing process
- Tissue harvesting (Schedule 1)

What are the expected impacts and/or adverse effects for the animals during your project?

Generally, the expected adverse effects are minimal for all protocols and this is because we are interested in early inflammatory reaction characterised by the infiltration of immune cells called macrophages. The duration of the experiment following induction of injury (general inflammation in protocol 2; kidney inflammation in protocol 3; skin healing in protocol 4) is relatively short (7-18 days), which in our hands do not cause any significant adverse effects to the animals other than transient discomfort.

Specifically, for the kidney inflammation protocol (Protocol 3), the animals are usually killed by Schedule 1 before they develop any renal failure. There is no pain nor discomfort associated with this protocol through the total duration (7 days generally). Similarly, the incidence of wound infection (protocol 4) is minimised by the use of appropriate aseptic surgical techniques and we have not observed any pain nor discomfort in the animals throughout the healing process (14-18 days).

Most importantly, in all protocols, treatments are applied to seek from protection from inflammation (protocol 2 and 3) and to accelerate the healing process following wounds (protocol 4). This strategy has an overall beneficial impact for the animals reducing further the transient discomfort they may experience.

Our protocols have been designed to reach a moderate level of severity at the maximum. Every animal undergoing recovery surgery will receive adequate and timely painkillers to reduce pain or discomfort after the procedure. Signs for infections will be dealt by applying the appropriate treatments and immune-compromised animals will be held in designated facilities. Animals will be humanely killed using approved methods at the end of the protocol. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates significantly will be humanely killed.. All the procedures will be carried out while working closely with experienced animal care staff. All the protocols proposed in this licence underwent considerable refinement and some protocol (e.g. kidney inflammation in rats) have been used for >20 years, where there is cumulative expertise in all the procedures involved. There are no new (or previously not tested) protocols proposed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The protocol 1 refers to the breeding and maintenance of genetically modified animals. In this protocol the expected severity is mild and less than 5 percent of the animals are expected to experience this severity. Protocol 2 aims to measure immune and inflammatory responses in rats and mice. Half of the animals will experience moderate and the other half mild severity. Protocol 3 is set to induce kidney inflammation and all animals are expected to experience moderate severity. Finally, Protocol 4 is proposed to study wound healing in the skin and all animals are expected to experience moderate severity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of animals is essential for our work, since it is only by study of the intact animal that we will be able to work out how inflammatory disease is caused and what therapeutic approaches might be of benefit. We also use cell culture experiments, where possible, to address specific questions about cellular mechanisms of disease, but this cannot reproduce the complexity of the whole animal **Which non-animal alternatives did you consider for use in this project?**

We also use cell culture experiments, where possible, to address specific questions about cellular mechanisms of disease, but this cannot reproduce the complexity of whole animal tissues and organs. We have parallel projects that use human tissue material where possible. These projects identify targets (genes, gene products, soluble factors) that we ultimately target using animal models. We take advantage of integrative approaches in human tissues (multiple and diverse technologies) to prioritize biological targets.

Why were they not suitable?

Although some human tissues can be accessed during surgery, animal models are valuable in offering a 'treatment' component - the key reason for using refined models of inflammatory disease. Our research is very much focussed on generating robust and reliable hypotheses that can only be tested in animal model as the final confirmation of a potential therapeutic approach.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The applicant's previous licence included the same protocols that are proposed in this replacement application. Experiments conducted under the previous licence for a period of 5 years, allowed us to estimate the number of animals that are required in each experimental protocol. Briefly, their number is determined by:

- good knowledge of the protocol that can provide a conclusive result that will be sufficient for data sharing (e.g. publication or submission for funding applications)

- the existence knowledge on previously published worked by us and other scientists (publications, publicly available databases).

- in vitro models (those that do not use any living system) that may complement the in vivo (involving living organisms) usage (where applicable).

- robust experimental design refined efficiently to provide the most comprehensive information

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

1. The Experimental Design Assistant (EDA) from the The National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) for appropriate statistical analysis and visualization of the project.

2. Peer-review of experimental design and statistical analysis by the Medical Research Council. The MRC has an online tool that includes the Reduction as part of the Research Project applications - the power calculations (statistical analysis that are made prior to undertaking research - this determines the number of animals required for the proposed experiments) are submitted by a collaborative effort with a medical statistician and the Reduction plan of the Research Project gets peer-reviewed with feedback provided to the main applicant.

3. Collaboration with biomedical statisticians and data scientists.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Efficient breeding. Only the necessary animal numbers provided by power calculations will be considered through an efficient and reductive breeding strategy.

Pilot studies will provide preliminary data on future experiments. For instance, we have determined that the usage of 3 rats as control and 3 as 'treated' animals gives enough statistical power to determine whether a potential treatment is effective. This minimal and highly informative approach will be applied where necessary.

Sharing of tissue. Tissues are shared between collaborators in the UK using similar protocols. Where necessary, we will use these tissues to further reduce the animal numbers required in our own protocols.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This application includes both rat and mouse models of inflammatory diseases, since each species has advantages for certain studies. One advantage of mouse models has been the availability of a wide range of genetically modified mice which can be used to determine the effect of specific genes on the disease model being studied. However, there have been major advances in rat genetics over the last

few years, and several genes involved in macrophage-dependent inflammatory disease in the rat have been shown to be relevant in the related human disease. Also, recent advances in techniques mean that specific genes can be deleted in rats in order to study their relevance in particular disease models. We therefore wish to generate and breed genetically modified rats in order to investigate the more accurate models of human disease that can be produced in the rat as compared with the mouse.

Because of their reproducibility, rapid onset, the mice and rats models studied in this licence cause no lasting harm to the animals. For instance, the model of kidney injury in the rat is obtained by using a method that causes the least pain, suffering or distress. This is because of the genetic background of the animals which make them susceptible to disease. Hence, we use this method preferentially in susceptible animals and cause kidney injury in an efficient, rapid and reproducible manner.

Because of the application of Replacement, Reduction and Refinement (3Rs) in all of our studies involving inflammatory disease in experimental animals, we assess the outcome of the experiments by using laboratory techniques, at a relatively early stage of disease such that no animals suffer from the clinical effects of prolonged inflammation (i.e. kidney or lung failure). Any animal which becomes unwell during the course of the experiments, for any reason, will be humanely killed.

Another aspect of refinement is the usage of recently available data-rich techniques such as next generation sequencing methods. In recent years, we have collected and analysed these kinds of data from multiple tissues from single animals in various models of inflammatory disease. We have established a data analysis pipeline which allows identification of molecular targets using computer-based methods. This minimises experimental approaches. It also makes any experimental approach focussed and with a clear hypothesis.

Why can’t you use animals that are less sentient?

Rats, and in particular the certain strains, are genetically susceptible to inflammatory disease. Similarly, mice have been widely used in immunology and biomedical research because of the possibility to mimic human conditions and modelling similar pathologies. Because of their unique susceptibility to disease and 3R principles that can be implemented, these species are the most appropriate ones for the proposed protocols.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Because of the application of 3 Rs in all of our studies involving inflammatory disease in experimental animals, we assess the outcome of the experiments by studying tissue samples under a microscope, at a relatively early stage of disease such that no animals suffer from the clinical effects of prolonged inflammation (i.e. kidney failure or skin inflammation). Any animal which becomes unwell during the course of the experiments, for any reason, will be humanely killed. We will increase monitoring, post-operative care and pain management in all protocols.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) are a checklist of recommendations to improve the reporting of research involving animals – maximising the quality and reliability of published research, and enabling others to better scrutinise, evaluate and reproduce it. The new ARRIVE 2.0 guidelines have been recently published in a scientific journal so that they are now accessible to the wider public. These build on previous guidelines published by the NC3Rs in 2010 and set out to address comprehensive improvements in reporting.

The “ARRIVE Essential 10” that are the basic minimum to include in a manuscript to enable readers and reviewers to assess the reliability of the findings, and the complementary “Recommended Set” that provides context to the study. The aim is for the scientific community to focus initial efforts on the Essential 10, with the recommended set subsequently adopted as best reporting practice.

Another aspect of refinement is the usage of recently available data-rich techniques such as the fast growing advances in sequencing methods. In recent years, we have collected and analysed these kinds of data from multiple tissues from single animals in various models of inflammatory disease. We have established a data analysis pipeline which allows identification of molecular targets in silico, using exclusively computational methods. This minimises experimental approaches by offering a hypothesis-driven approach.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) is an independent scientific organization in the United Kingdom (UK) that was set up by the government

to lead the discovery and application of new technologies and approaches that minimize the use of animals in research . They help the research community use the latest science and technology to replace animal studies, providing new approaches for biomedical research . Where the use of animals is still required, they support researchers to design the best experiments so that their methods and findings are robust and reproducible. They provide information and guidance on the latest knowledge to improve laboratory animal welfare.

Home Office

NON-TECHNICAL SUMMARY

174. Studying microbial pathogenesis to cure infections

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

Infectious diseases, Microbial metabolism and virulence, Host response, IVIS, mouse infection models

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This study aims to define bacterial and host factors active during infection to develop and test therapeutic and prophylactic compounds.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Microbial diseases pose a large threat to public health and they continue to be an important cause of death and suffering around the world. Antimicrobial resistance continues to be a major problem, and some of the existing vaccines are not very effective. Therefore, we urgently need effective vaccines and new therapeutic strategies. One of the well-established ways to identify new anti-infective targets and strategies is to study the 'behaviour' of microbes during infection. We hypothesis that by using different metabolic pathways in different host tissues, bacteria acquire distinct phenotypes. Once the critical microbial metabolic pathways for *in vivo* survival are identified, we can target them pharmaceutically to produce much-needed anti-infectives.

There is a severe lack of knowledge on how microbes survive in different host tissues for example for bacteria that are going to be used commonly in this study such as *Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Unless we have a detailed understanding of microbial survival strategies in host, the efforts to combat infectious agents will be a much harder task. So far in-host survival mechanisms have been studied using a small number of strains of individual microbial species but there is a significant variation in the ability of different strains of same species to cause disease due to the differences in their genetic background. Moreover, so far there has been little attention to the microbial response to the *in vivo* environments or how external environments effect the *in vivo* behaviour of microorganisms. In host tissues, individual species of microorganisms do not exist in isolation but rather interact with the existing microbiota. However, there is incomplete understanding of the *in vivo* interactions of different microbial populations. Infections do not occur solely due to the microbial factors. Host response is an important factor for the occurrence and progression of infectious diseases. Despite this there is an incomplete understanding of the host's response to infections and how microbial metabolism shapes host response during infection.

The study of microbial metabolism *in vitro* and *in vivo*, will allow the identification of essential microbial metabolic pathways as well as the impact of microbial products in formation of host response during acute and chronic infection. Consequently, the data generated in this study can then be used for developing effective anti-infectives, and enable a detailed understanding of pathophysiology of infectious diseases affecting human health.

What outputs do you think you will see at the end of this project?

Main outcomes of this project will be a detailed understanding of pathogen biology, host response to infection, and identifying and validating anti-infective targets. In this study, we will test hypotheses relating to how various microbial species of medical importance cause disease, and identify the host factors important in response to microbial agents. By using the models of pneumonia, meningitis, septicaemia, and colonisation, we will generate complementary data for a holistic understanding of microbial and host factors important for colonisation and virulence. This study will generate new information on infectious disease biology and can potentially reveal commercially exploitable data by testing anti-infective compounds. In the projects after this, such anti-infective compounds can be developed into drugs and/or vaccines.

Who or what will benefit from these outputs, and how?

In the short term, users and beneficiaries of the data will include the academic community, particularly those interested in microbiology, genetics, and infectious diseases. Dissemination of results to the wider scientific community will occur throughout the project in the form of conference presentations, and publications after IP considerations. We envisage that 18 months after the start of the project, publishable data will be generated, and these data will be shared with the scientific community by publications in high impact journals. In addition, the data generated during this project will be disseminated via presentations at local, national and international conferences. As well as the scientific community, notable outcomes of the project also will be communicated to the general public. Public awareness of the research will be promoted through the institutional press office. The potential commercial development of our discoveries will no doubt require additional resources. We do not anticipate that antimicrobials will be generated within the lifetime of this project. However, this project will reveal viable candidates for anti-infective development which can be further developed in future studies. Any commercial potential of our findings will be exploited. Anti-infectives developed by data generated in this study will be beneficial both for human and animal health.

How will you look to maximise the outputs of this work?

I will maximise the outputs from this work by using my extensive network of collaborators. I collaborate with overseas and UK colleagues. My collaborations have been productive and led to publications in high impact journals and research grant awards. Combining my efforts with their expertise will surely increase the quantity and quality of my data.

Species and numbers of animals expected to be used

- Mice: 5040

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will be using adult mouse in this study. It is by far the most widely chosen animal for the study of infection and host responses, providing very reproducible models of infection. The genetics of the mouse is the subject of a global research effort, meaning that the data generated in this study can be interpreted in detail. Furthermore, there are many in-bred mouse strains available as well as strains with defined mutations for the study of disease processes. Additionally, the extensive background of existing experimental murine data is a very valuable resource.

Typically, what will be done to an animal used in your project?

We will use four protocols in this study. With the first two protocols, we will initiate either the models of acute- (Protocol 1) or chronic infection (Protocol 2) using the microbes that make us sick. These models will be an extremely valuable tool in understanding the mechanisms by which the microbes cause disease in humans. With Protocol 3, we will test the safe doses of selected compounds that can treat infectious disease or those with an

impact on our immune system (Protocol 3). This step will allow us to determine the non-toxic dose of study compounds against the infectious agents, and prevent unnecessary suffering of animals. Protocol 4 will be used to produce antibody. This protocol will provide the required tool to understand how our immune system functions during infectious disease.
Each protocol has several mandatory and optional steps. The optional steps are required, for example, either to establish certain infection models or to assess the impact of selected compounds. Depending on the objective of each experiment, these optional steps increase the data output per animal reducing potentially the number of animals and/or refining our protocols. It should be noted that animals will not go through each step in these protocols.
In Protocol 1, 2, 3, and 4, the maximum number of steps including the mandatory euthanasia step, the animals will go through 5, 5, 4, and 3 steps, respectively. At the end of each protocol, mice will be killed humanely by schedule 1 or non-schedule 1 method. Typically, we will administer infectious agent to follow the clinical signs, and evaluate host immune response (Protocol 1 and 2), or administer antiinfective agent to determine the toxicity or efficacy of compounds under study (Protocol 3 and 4). We will obtain tissue samples such as blood or peritoneal fluid to assess the progress of infection and evaluate host response (Protocol 1-4). These processes will inevitably inflict pain and discomfort to the mice. However, we will do every effort to reduce the pain and suffering by closely monitoring animals and setting up humane endpoints using our robust scoring system, through use of anaesthetic and analgesics, and continuous application of good practice in the field.
Protocol 3 and 4 are designed to evaluate the dose for therapeutic and immune-modulatory compounds and antibody production, respectively, and these animals will not be infected. Data generated in Protocol 3 and 4 will be very useful for Protocol 1 and 2, where we plan to administer the non-toxic concentrations of compounds under study or evaluate the protective ability of selected compounds. Protocol 1 and 2 aim to initiate acute or chronic infection, respectively. They evaluate the role of selected microbial products and compounds on the outcome of infection or enable us to study the pathophysiology of the infectious disease process. This understanding can lead to the development of drugs and vaccines in future.
The duration of each protocol will vary. For example, Protocol 1 will be up to 7 days, while Protocol 2 to create chronic infection can be up to 90 days. Protocol 4 can be up to 90 days. The therapeutic dose setting experiments can be as short as 24 hours or extended over several weeks to evaluate the longterm toxicity (Protocol 3).

What are the expected impacts and/or adverse effects for the animals during your project?

In the project experiments, the animals may experience pain and discomfort due to (i.) the administered compounds, (ii.) infectious agent, and (iii.) procedures that will be applied.
(i.) Adverse effects due to the administered compounds:

Administration of immunising agents and adjuvants can drive moderate inflammatory responses in the tissue, which could be prolonged. Animals may manifest their discomfort by changing their routine eating and drinking habits or by altering their interactions with other mice.
X-irradiation of mice can leave them susceptible to infection as the bone marrow reconstitutes their immune system. Radiation renders mice susceptible to opportunistic infections, leading to clinical signs such as diarrhoea. Particularly, *Pseudomonas aeruginosa*, can translocate through the gut in irradiated animals and this can lead to bacteraemia and sepsis. Animals may develop dry skin due to radiation. The animals may rarely experience post-irradiation lethargy.
Mice receiving inducing, labelling, antibiotic or immunomodulatory active agents have the potential to respond negatively although most treatments will be based on previous successful delivery (literature or documented historical data sets), any mouse undergoing these treatments will be monitored.
(ii.) Adverse effects due to infectious agents:

For example, after infection with respiratory pathogen *Streptococcus pneumoniae*, which will be frequently used in this study, mice move progressively through piloerection, hunched, lethargic and moribund before death. The end-point in this study will be when the animals become lethargic, being immobile unless prompted when they

will be humanely killed by a Schedule 1 method or immediately placed under terminal anaesthesia to obtain cardiac blood. In order to minimise their suffering, animals will be infected at a time of day and week when they will be able to be inspected at their most critical times, and treatments will be administered to animals not later than the early lethargic stage.
In chronic infection, mice can lose weight and when the weight loss reaches 20% relative to the weight of age-matched controls, the mice will be culled by the Schedule 1 or non-schedule 1 method.
(iii.)Adverse effects due to the procedures: During multiple dosing, administration of agents may cause discomfort (e.g. local injection site reaction) but the likelihood of such an event is low (<10%).

Venepuncture can lead to excessive bleeding. Animals may feel pain and discomfort during tissue sampling.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Up to 96% of animals may experience moderate severity limit
.
Up to 4% of animals may experience mild severity limit
.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

To achieve the project aims and objectives we will employ a comprehensive set of in vitro and ex vivo assays. However, to be able to assign a role to different microbial metabolic pathways in vivo, and to study the impact of these pathways in interaction with host molecules, the use of animals is essential for complete understanding. While in vitro and ex vivo experiments are useful, they cannot mimic the complexity of in vivo environment, and only through the inclusion of animal models, we can fully understand the key microbial metabolic pathways at different stages of infection.

Which non-animal alternatives did you consider for use in this project?

We considered replacement alternatives but the total replacement of animal models for this project is not possible. Whenever possible, we will use replacement alternatives. For example, when we do not see the impact of the efficacy of a tested chemical in in vitro and ex vivo models, we will not test them in vivo. The number of anti-

infective compounds to be tested in animal models will be reduced based on the structure-function studies. If the compounds have the same mechanism of predicted activity, only a few representative compounds will be tested. Similarly, we will use initially ex vivo and in vitro assays before in vivo models. This so-called “stepwise testing strategy” is recognised by FRAME as a way of reducing animal numbers and suffering. Where appropriate, we will also use the *Galleria mellonella* model of infection and ex vivo models, e.g. respiratory and brain ciliated epithelial tissue models instead of rodents.

Why were they not suitable?

As discussed above, the full complexity of bacterial metabolism and its impact on host-pathogen interaction cannot be understood fully using in vitro and ex vivo models. Bacteria are versatile entities and can manifest distinct phenotypes under different environmental conditions. We cannot assume that the relative importance of metabolic pathways will be the same in vitro and in vivo. Bacteria encounter very complex and fluctuating environment in host tissue than in vitro and creating this environment in vitro is currently not possible.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of mice needed for each experiment has been calculated in collaboration with statisticians and by using the NC3R’s Experimental Design Assistant tool. The number of mice that will be required in our proposed experiments will be the minimum number needed to achieve the project objectives. Information from previous studies has been useful in predicting the number of mice in each experiment required to determine the answer to the important scientific questions using as few mice as possible. We will continuously monitor group sizes in the light of data generated throughout the course of the project and will introduce the required adjustments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To reduce the number of animals being used in this project, we sought advice from local statistician and also made use of NC3Rs' Experimental Design Assistant. In addition, I sought advice from colleagues who have long term experience in the use of animal models of infectious diseases.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To prevent the wastage of a large number of animals, before doing large scale experiments, we will do small scale pilot studies to decide how best to conduct a large-scale experiment. Moreover, if the gender is not a variable in the hypotheses we test, this study will use both male and female rodents. In addition, we will store tissues for future use and will share tissue samples with other colleagues who have similar research interest.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse models of infection will be used. It is the most frequently used animal for the study of infectious disease biology. There are many in-bred mouse strains, as well as strains with defined mutations for the study of disease processes. Furthermore, the genetics of the mouse is the subject of a global research effort, including making knock-out strains covering every mouse gene. Additionally, the extensive existing experimental data with the mouse is a very valuable resource.
Our experience in working with animal models of infectious diseases show that as the disease develops animals gradually move through piloerection, hunched, lethargic and moribund before death. Each stage can be finely rated as 1+ or 2+. This scoring scheme has proved remarkably reliable and only on very rare cases is an endpoint missed, which allowed us to predict the stage of infection and prevent the suffering at the right time during the course of infection without significantly compromising the quality of scientific data. The suffering will be minimised by evaluating the disease sign scores regularly. In addition, we will refine our models through the use of in vivo imaging technologies.

Why can't you use animals that are less sentient?

Wherever possible we will use *Galleria molenella* (greater wax moth) model for pre-screening anti-infective compounds and our bacterial strains. This model has been a highly informative tool for the study of infectious disease biology. Although it is useful, it cannot mimic the complexity of mammalian tissue. Infection process in this model is initiated by injecting the inoculum into the haemocoel of *G. mellonella* larvae. This means that common natural infection routes that can be created in a mammalian host, such as inhalation or oral routes, cannot be generated in this model. Moreover, unlike rodents, *G. mollonella* does not have adaptive immunity, which hampers studies of host-pathogen interaction.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In our models, we know the approximate timing of disease sign development. Once the disease signs set, generally 24 h post-infection, we will increase the periodicity of monitoring. When appropriate, to alleviate pain, we will administer pain killers after consultation with the named veterinary officer. The usual procedure under this licence is to kill any animal as soon as it becomes very lethargic stage when they are immobile unless prompted to prevent suffering.
Throughout this project, we will refine our methodology based on our results, based on relevant publications of other scientists, and the guidelines issued by the Home Office and NC3Rs. Animals will be infected at a time of day and week when they will be able to be inspected at their most critical times in order to minimise their suffering. Recently, I developed efficient microbial bioluminescent strains, which will be used during the course of this project. These strains will help me refine my models and reduce the number of animals used in this study. For example, by relating the number of bacteria to the light intensity, we may be able to stop the course of infection at an earlier stage. Although these techniques involve additional procedures, the quantity and quality of data can be markedly increased to allow more detailed analysis of disease progression and timing of termination of experiments. The irradiation administered in a single dose can compromise intestinal mucosa and so half doses administered 2 hours apart reduces potential adverse effects and improves outcome.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow NC3Rs guidelines to ensure experiments are conducted in the most refined way. We will subscribe to the electronic version of the monthly newsletter of NC3Rs. Also, the Nuffield Council on Bioethics has relevant published work concerning animal welfare and refinement of animal experiments. We will seek advice from the experts on 3Rs based in NC3Rs and will regularly attend and present our work at scientific meetings.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will follow the guidance issued by the NC3Rs, attend relevant symposia and conferences organised by Microbiology Society and Laboratory Animal Science Association, and through relevant published data in the field. At NC3Rs web site valuable guidance on various 3Rs topics is available including on animal welfare, experimental design for animal experiments, and good animal housing and husbandry practices. We will seek advice from the experts on 3Rs based in NC3Rs, and will regularly attend and present our work in scientific meetings. The advances in 3Rs will be implemented in collaboration with my colleagues.

Home Office

NON-TECHNICAL SUMMARY

175. Targeting Fibroblast Heterogeneity, Plasticity and Functions in Pancreatic Cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Pancreatic cancer, Tumour microenvironment, Fibroblasts, Cancer therapies, Cellular cross-talks

Animal types	Life stages
Mice	embryo, pregnant, adult, juvenile, neonate, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to understand how different populations of non-cancerous cells, called fibroblasts, support pancreatic cancer progression to identify new combinations of treatments that block the tumour promoting cross-talks of cancer cells and fibroblasts.
A retrospective assessment of these aims will be due by 24 January 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Pancreatic cancer is highly lethal. Drug resistance is one of the main reasons of pancreatic cancer lethality and is largely caused by non-cancerous components, called stroma, that comprise up to 90% of the tumour mass. Within the stroma, fibroblasts are the most abundant cell population and promote drug resistance and cancer growth. No current therapy effectively targets these cells. Although previous studies provide insights into the wide range of fibroblast cells (i.e. fibroblast heterogeneity) in pancreatic ductal adenocarcinoma (PDAC), the extent of this heterogeneity, the roles of distinct fibroblast subtypes and how to selectively target them remain unclear.
The role of cancer-associated fibroblasts (CAFs) in modulating tumour progression is emerging as a strong player in therapy resistance, metastasis formation and poor prognosis. However, whereas the biology of pancreatic cancer cells have been extensively investigated, the roles of pancreatic CAFs are largely unknown. Evidence suggests that distinct fibroblast groups (i.e. subtypes) play different roles in the progression of pancreatic cancer, and that targeting them individually may lead to disparate outcomes. We propose that strategies using combinations of drugs that target tumour-promoting fibroblast populations could be used in the future for pancreatic cancer treatment.
It is therefore important to deeply characterise different fibroblast subtypes to understand how to target them. The strategies proposed herein will directly address knowledge gaps that could have significant therapeutic implications. Notably, the emerging understanding that fibroblast heterogeneity is not restricted to pancreatic cancer indicates that our findings could be applied to other tumours.

What outputs do you think you will see at the end of this project?

The completion of this project will significantly expand the knowledge about fibroblasts and the tumour microenvironment (i.e. the arrangement and characteristics of the different cells in the tumour) in pancreatic cancer and will potentially identify new ways to better group patients for different treatments. Overall, the investigation of fibroblast biology may lead to new strategies to treat and detect pancreatic cancer. Additionally, the publications originating from our studies could inspire future work in various laboratories across the world. As it is emerging that fibroblast heterogeneity is present in other malignancies and inflammatory conditions, our findings could provide useful insights to other cancer fields and be of interest to the broader scientific community.

Similarly, we will generate and make available to the scientific community new mouse models that could allow to investigate fibroblast functions in other malignancies, diseased states and normal tissues, and could thus be important for the scientific advancement in other fields.

Who or what will benefit from these outputs, and how?

In the short term (2-3 years), the scientific community will benefit from our discoveries through peer reviewed (i.e. assessed by other academic experts in our field) open-access (i.e. available to everyone without a journal subscription/fee) publications and open-access, not yet peer-reviewed pre-prints originating from our work. This will promote further research and rapidly advance our and others field. Papers published on both of these platforms will also be available to the non-scientific community, so that everyone could be informed, if interested, about our scientific progress. Additionally, we will present our work at conferences and workshops. We will also engage the non-scientific community in public events. We will also host college students over the summer, to train and inspire the next generation showing them our research progress and models.
Our main long-term goal is to deeply understand the mechanisms regulating tumour heterogeneity to develop strategies that would benefit patients. Although this output will not be rapidly evident following our work, as it will need to be evaluated in clinical trials, our studies will be a first step towards that direction.
Eventually, pancreatic cancer patients could benefit from the design of selective combinations of drug treatments resulting from our research and specific for their particular tumour.

How will you look to maximise the outputs of this work?

We aim at publishing at least one article per objective. When possible, we will also submit these works before peer-review, as pre-prints, so that the findings of these studies will more rapidly advance research and will also limit potential duplications and unnecessary mouse work in other laboratories. Publications of unsuccessful or non-high impact approaches that would spare unnecessary duplications could also be deposited as pre-prints.
As similarities between fibroblasts in pancreatic cancer and fibroblasts in other cancer types are emerging, we also aim at establishing collaborations with other laboratories.
We are also planning to share with other laboratories the new mouse models that we will generate. Of note, these new mouse models could be used in the future to study the roles of distinct fibroblast subtypes in other cancer types, inflammatory conditions, and normal tissues.

Species and numbers of animals expected to be used

- Mice: 18300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We aim to deeply investigate the biology of fibroblasts in pancreatic ductal adenocarcinoma (PDAC), while keeping in sight a potential clinical output. Using the best models of PDAC is, therefore, a priority. We will use mice for the proposed study. Mice are commonly used as an animal model of PDAC as, more than any other model, they recapitulate the progression of the human disease, the crosstalk between cancer cells and stromal cells, such as fibroblasts, and the response to therapies. Finally, mice are required to test the efficacy of potential anti-cancer therapeutics prior to their testing in clinical trials. For example, the KPC mouse model remains the gold-standard for studying PDAC biology, progression and therapeutic response, as numerous parallels can be made between KPC tumours and the human disease.
This is possible because the KPC mouse model has been genetically altered to express in its pancreas the key genetic mutations that occur most frequently in human pancreatic cancer, hence it develops pancreatic tumours which accurately reproduce the characteristics of the tumours that develop in humans.

Our protocols for 1) breeding and maintenance of genetically altered mice, 2) breeding and administration of substances to genetically altered mice, and 3) production/maintenance of genetically altered mice that develop autochthonous (i.e. spontaneously arising) pancreatic tumours (e.g. KPC mice) will include various life stages. For other protocols, e.g. drug studies with genetically altered mice bearing autochthonous pancreatic tumours and transplantation of pancreatic organoids (i.e. three-dimensional cultures of pancreatic cancer cells) into the pancreas (i.e. orthotopically), we will only use adult (and aged) mice, as we aim to investigate tumour biology and tumour response to treatments.

Typically, what will be done to an animal used in your project?

Some mice will be administered drugs in the food or water while pregnant or before and after birth, for up to 6 months.
Some mice will develop pancreatic tumours, either spontaneously if genetically engineered mouse models (GEMMs) or following transplantation of organoids into the pancreas, in some cases after having aged. Some of these mice may be imaged (for example by ultrasound) to detect the presence of a tumour in the pancreas and humanely killed, prior to analysis of tissues.
Some mice will develop tumours and will be imaged by ultrasound and will be administered drugs (i.e. enrolled in drug studies). Mice enrolled in drug studies will be typically alone in the cage to decrease the experimental variability, which will overall decrease the number of mice needed. Mice will be then humanely killed, prior to analysis of tissues.
For genetically altered KPC mice, spontaneous pancreatic tumours develop at 3-6 months of age. Following tumour formation, the mice can survive approximately for up to 1-2 months. For transplantation models, pancreatic cells will be typically injected into the pancreas at 6-8 weeks of age, unless performed in aged mice around 16-18 months of age. Depending on the cell line and number of cells transplanted, tumour presentation typically occurs between 1 (more common) and 12 (rare) months from surgery. Following tumour formation, the mice can survive approximately for up to 2-3 months.

What are the expected impacts and/or adverse effects for the animals during your project?

Both genetically altered KPC mice and transplantation models bearing pancreatic tumours recapitulate clinical signs observed in pancreatic cancer patients, including loss of appetite, weight loss, inactivity and ill health. Additionally, KPC mice can develop other clinical signs typical of advanced pancreatic cancer in patients, including ascites (i.e. accumulation of fluids in the abdominal cavity) and bowel obstruction (when the presence of the tumour is blocking a part of the intestine). These symptoms usually occur at a late stage of tumour development in the mice and will normally only be present for 12 weeks prior to reaching a humane endpoint. During their life time, KPC mice also often develop facial and anal papillomas (i.e. benign epithelial tumour growths) that do not cause discomfort, if not infected, unless they obstruct vital functions, such as eating, drinking or defecation.

Administration of substances via injection or oral gavage (i.e. with a feeding tube that reaches the animal stomach) will cause recurrent (daily or weekly), transient (10-15 minutes) discomfort and distress, due to the restraint and procedure required. Administration of substances to the food may also cause discomfort and loss of appetite due to poor palatability, leading to transient weight loss.

Ultrasound imaging could cause transient distress and discomfort normally associated with the anaesthesia. Ageing mice may exhibit phenotypes associated with ageing. Common age-related clinical presentations, which are not expected to cause adverse suffering and can be managed are: i) skin lesions, such as loss of hair; ii) ocular lesions, such as conjunctivitis; iii) hearing loss; iv) reduced mobility; and v) body weight changes. Additionally, adverse age-related clinical presentations may be: i) visible or palpable masses, including tumours; and ii) prolonged body weight loss.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Overall, <2.5% of genetically altered KP mice (i.e. parental mice of the KPC mice), <2.5% transplantation models (i.e. models in which pancreatic cancer cells are injected in the pancreas of mice), <5% genetically altered KPC mice may be in a severe (in terms of level of pain, suffering and distress) category. The majority of mice will experience a moderate severity, and the remaining will be in mild or sub-threshold (i.e. even lower than mild in terms of level of pain, suffering and distress) categories.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 24 January 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Although recent improvements have occurred in the development of novel laboratory platforms for the study of the tumour microenvironment, animals remain the most informative model to recapitulate the cross-talks of cancer cells and fibroblasts observed in patients, because only a tumour in a living animal, such as a mouse, can exhibit all the complex features of a human tumour. To carry out impactful and meaningful research that could lead to the development of new therapies, it is therefore essential to use animals for the investigation of cancer/fibroblast cross-talks.

Which non-animal alternatives did you consider for use in this project?

As an additional approach, we will employ research alternatives that do not involve the use of animals. In particular, for some of the biological questions we aim to address, we will employ three-dimensional pancreatic organoid/fibroblast co-cultures.
Although the use of these co-cultures will reduce the number of mice employed in this project, it cannot completely substitute for it as, to date, no system can accurately recapitulate the fibroblast cross-talk with pancreatic cancer cells and the surrounding microenvironment as well as mouse models.
However, we are also actively working on optimising our current co-cultures by including additional cell types, such as immune cells, with the hope to develop more meaningful laboratory models for the study of pancreatic cancer biology.

Why were they not suitable?

Although our co-cultures will allow to reduce the use of animals, they cannot entirely replace the use of these models as they do not fully recapitulate the tumour microenvironment of pancreatic cancer.
Additionally, prior to testing our findings in clinical trials, the relevance of our findings will have to be evaluated in mouse models of pancreatic cancer that have been shown to largely recapitulate the human disease (e.g.

transplantation mouse models and genetically altered KPC mice).
A retrospective assessment of replacement will be due by 24 January 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These numbers are an approximation as the effective size will depend on the results obtained from the pancreatic cancer organoid/fibroblast co-cultures and initial smaller-scale (i.e. pilot) studies, when relevant. Indeed, animal usage may be reduced by performing pilot studies (with 4-5 animals) prior to the full studies with a larger number of mice. If biological and statistical significance is reached in a pilot study, a full study may not be required and this will reduce the number of animals used.
Additionally, the results gathered from experiments performed in the co-cultures will inform the design of the animal studies.
For transplantation models of pancreatic cancer, the cohort sizes have been initially determined based on our previous studies. Previously, n=5-10 per group has been sufficient to reach biological and statistical significance.
Similarly, for therapeutic studies in genetically altered mouse models (e.g. KPC mice), we have performed pilot studies to characterise growth rate and variability of tumours to inform subsequent studies. Previously, n=5-10 per group has been sufficient for monitoring the anti-tumour effect of drugs. Finally, based on our experience, for survival studies (i.e. studies in which animals are humanely killed only when they show severe clinical signs at humane enpoints), 10-12 mice per cohort have been sufficient to reach biological and statistical significance. Moving forward, we will re-assess these numbers when more information will become available with our future studies. To do this, we will work closely with our Bioinformatics core facility who will provide advice on the study design and statistical analysis to determine the number of animals needed.
Additionally, our Bioinformatics core facility uses a variety of statistical methods to analyse different types of datasets.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

At all times, we attempt to reduce, replace and refine our animal models. We are committed to limit the use of animals in our research. To this end, when scientifically meaningful, instead of using genetically engineered mouse models (GEMMs), we will establish pancreatic transplantation mouse models in which pancreatic cells or organoids are injected into the mouse pancreas. These models will reduce the number of mice needed to address our experimental questions, as they do not need the breeding required to generate the GEMMs. Although useful, these transplantation models cannot entirely replace GEMMs, such as KPC mice, as they bypass the early stages of tumour formation. The combination of both mouse models will be necessary for deeply understanding pancreatic cancer fibroblast biology and for evaluating new therapeutic strategies.
To further reduce the number of animals in our research, whenever possible and scientifically meaningful, we will use co-cultures of previously established murine and human pancreatic cancer organoids and fibroblasts.
These analyses will help indicate how and whether an animal study should take place.

Additionally, each therapeutic study will include all necessary controls, thus increasing the reproducibility and robustness of the study, and decreasing the variability and, therefore, the number of mice per group.
Additionally, we will employ ultrasound imaging to reduce the number of mice used in drug studies. Indeed, this technique allows to identify, and exclude, mice without pancreatic cancer or mice with other pathologies. Additionally, as the time needed for the development of a tumour is quite variable across animals (between 3-6 months), the use of imaging allows to start treating mice with similar tumour sizes (6-8 mm in diameter), reducing the variability during studies and therefore, reducing the number of mice needed.
Finally, imaging techniques allow to follow tumour growth at different time points, reducing the number of animals required to observe tumour growth at various stages.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies (with 4-5 animals) will be performed to characterise tumour growth rate and experimental variability to inform the design of subsequent studies and also to potentially reduce the number of animals used. Indeed, if biological and statistical significance is reached in a pilot study, a full study may not be required.
For genetically altered mouse models, whenever possible, we will maintain parents already with the required phenotype to reduce the overall number of mice needed, as all mice born from such breeding strategies will have the required genotype. Whenever this will not be possible, mice carrying inappropriate alleles will be humanely killed.
Although we do not expect surplus animals produced from our breeding strategy, if more mice than what we can use are born, the surplus may be transferred to other project licences to limit wastage. We will also share with other laboratories the tissues and organoid/fibroblast lines we will generate, to limit the use of additional mice elsewhere.
A retrospective assessment of reduction will be due by 24 January 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

For our research, we will use mice. In particular, we will use genetically engineered mouse models (GEMMs), such as KPC mice, and, when scientifically meaningful, mouse models with organoids/cells transplanted in the pancreas. These transplantation mouse models appear healthier than KPC mice (i.e. develop less clinical signs during tumour progression) and likely experience less distress and pain during tumour formation compared to GEMMs, as evident by reduced signs of ill health. Subcutaneous transplantation models of pancreatic cancer, which would further decrease the occurrence of clinical signs in the animals, and the associated pain, do not faithfully recapitulate the cancer/fibroblast interactions that are at the centre of our research, and we therefore cannot consider them as relevant and scientifically meaningful substitutes.

We will minimise suffering by adhering to best practice guidance, in accordance with the “Guidelines for the

welfare and use of animals in cancer research" by Workman et al. 2010, BR J Cancer and the National Centre for the 3Rs. Every procedure proposed has been refined in order to cause the minimum distress, pain and discomfort to the animals.

Why can't you use animals that are less sentient?

The mouse remains the most relevant species with the least sentience that we can use to predict the biology and therapy response of pancreatic cancer. In particular, KPC mice are the gold-standard for pancreatic cancer research, as they recapitulate many aspects of the human disease. Not only they recapitulate the characteristics of human pancreatic tumours, but they also recapitulate many cancer associated clinical signs typical of pancreatic cancer patients with advanced disease, including weight loss, jaundice (i.e. yellow skin colour), and ascites (i.e. accumulation of fluids in the abdomen).
KPC mice develop pancreatic tumours at 3-6 months of age and their analysis over the following few weeks is pivotal for the understanding of tumour progression and testing of novel therapeutic approaches.
Transplantation models of organoids or cancer cells in the mouse pancreas also largely recapitulate the human disease and associated clinical signs.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Environmental enrichment (i.e. material that is added in the cage to improve the psychological and physical well-being of animals, such as small tunnels) will be provided to improve animal welfare and promote the expression of species-appropriate behaviour and mental activities. More material that can be used to make a nest will be provided to mice that have been housed alone, for example, during a therapeutic study. Animals will be housed on an appropriate light/dark cycle with control of room temperature and humidity. Animals purchased and transferred to our facility will be allowed at least 7 days to adapt to their new environment and will be handled prior to any procedure or surgery to minimise their distress.

All mice will be monitored at least once a day by qualified, competent and trained staff. Post-operative care will occur following every pancreatic surgery (e.g. transplantation of tumour cells/organoids into the pancreas) and imaging procedure. Mice that show any clinical sign will be more closely monitored and, at all times, we will attempt to reduce any sign of discomfort, distress or pain (e.g. providing gel cups and food at the bottom of the cage, if possible). If long-lasting pain is experienced and no sign of improvement is observed from any measure that we will undertake, mice will be humanely killed.
We will use anaesthetics and analgesics during procedures that require physical restraint (e.g. ultrasound imaging), and during invasive procedures, such as pancreatic surgeries to minimize the animal distress and/or pain.
When mice are to be given certain agents in the diet, it is well documented that mice refuse to eat the diet for a few days due to its unpleasant taste. This results in a transient weight loss, which is typically recovered after a few days, when the animal starts eating again. However, if possible, to try limiting the weight loss by making the food more appealing, Nesquik or condensed milk may be added to the regular mashed diet. If this were to be the route of administration, mice would be exposed to Nesquik or condensed milk mashed food prior to treatment to acclimatise them to the diet. During the course of this licence, we will regularly monitor and try to reduce the percentage of mice that experience transient weight losses associated with this type of drug administration.
All protocols will be annually reviewed based upon records of severity kept under the Animals Scientific Procedures Act (ASPA) requirements to ensure that any new advances that can minimize the severity (in terms of pain, distress and discomfort) will be incorporated.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We are committed to following the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery in our surgical protocols. We will also follow guidelines in accordance with Workman et al 2010, Br J Cancer and the National Centre for the 3Rs, which are considered the best current practice. We will also regularly review and implement any updated guidance from the National Centre for the 3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We currently follow any update provided by the National Centre for the 3Rs and, whenever possible, we will implement any new advance identified in term of replacement, reduction and refinement. We will also attend conferences and keep up-to-date with the current publications on themes related to the animal work carried out in our laboratory (e.g. conferences on animal models). These approaches will allow us to be exposed to the newest advances in the field which, whenever scientifically meaningful, we will start implementing in our research.

A retrospective assessment of refinement will be due by 24 January 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

176. Targeting signalling pathways driving chronic inflammation and tissue damage in osteoarthritis and related musculoskeletal diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Zebra fish	embryo, adult, neonate, juvenile
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overarching aim of this project is to identify the key cellular mechanism mediating tissue injury/damage in musculoskeletal diseases particularly osteoarthritis. This will facilitate identifying druggable targets for targeted therapy.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In this Project, we aim to identify the upstream ubiquitination machinery mediating tissue injury inflammatory response. This is crucial for understanding the role of injury in initiating repair, and promoting pathological tissue damage. Knowledge of this new mechanism will define a pathway that could be targeted to reduce pathological injury responses in musculoskeletal diseases such as osteoarthritis which is crucial for understanding the disease behaviour and for designing new drugs for targeted therapy in osteoarthritis- a disease with no available disease modifying therapy.

As this early injury response may initiate wound healing, identification of this new mechanism may suggest new therapeutic approaches to delayed healing of wounds and fractures. Furthermore, investigating a new mechanism initiating inflammation may also give insights into inflammatory diseases of unknown aetiology such as rheumatoid arthritis, other rheumatological diseases, inflammatory bowel disease and many other diseases caused by chronic inflammation.

What outputs do you think you will see at the end of this project?

We envisage that our programme of work will lead to improved understanding of the genetic and cellular processes involved in tissue damage in response to mechanical injury. In so doing we hope to identify treatments that could be used to promote joint healing and repair in diseases like osteoarthritis.

Some of the outputs we expect during and at the end or the project include:

- New data that will be made discoverable via peer-reviewed publications, presentations at key meetings, deposited proteomics data in PRIDE, and through the publication of our datasets.
- New zebrafish transgenic lines: these will be available for zebrafish research community.
  Data that may be of use to the pharmaceutical industry for potential new therapies for musculoskeletal
- diseases [long-term]

Who or what will benefit from these outputs, and how?

Key groups that will benefit from findings from the outlined project include:

Scientists working on the project

Immediate beneficiaries of this project will be the scientists working on the project (PI, RA and PhD students). With the opportunity to apply new approaches and techniques with Zebrafish models there will be career

development opportunities. No procedures are undertaken for the purposes of training or development of staff - i.e. all procedures contribute to the scientific objectives. Researchers will be trained in the priority skills areas of whole organism physiology, quantitative skills (proteomic analysis), and interdisciplinary (particularly imaging).

Scientific Research Community

Academic researchers in several research areas will benefit from this work including those working on pathogenesis of osteoarthritis, musculoskeletal ageing, researchers in inflammatory diseases field, zebrafish research community and researchers in cell signalling and ubiquitination field.

Future Scientists

Our research group will work directly with student at GCSE and A-level through a work experience programme to enhance their understanding of scientific research and inspire career pathways in science.

Patients and wider public

Patients and the wider population will benefit from this research if we can identify novel pathways for future research and ultimately, targets for therapies to lessen the burden of diseases caused by tissue damage.

Pharma and Biotech companies

This work will identify drug targets that could be used to treat a huge unmet clinical need. It is likely that this will be of significant interest to pharmaceutical companies, large and small. We will develop strong collaborative links to enable commercialisation of any targets as they arise.

How will you look to maximise the outputs of this work?

We plan to maximise the outputs of this work through:

- effective collaboration with experts in the field of zebrafish mutagenesis as well as osteoarthritis research field which will extend our skill set and facilitate access to advanced technologies and approaches for improvements of our techniques and methods • dissemination of new knowledge at key meetings and conferences
- dissemination of work through press release via university media team publication and
- sharing of unsuccessful approaches.

Species and numbers of animals expected to be used

- Mice: 550
- Zebra fish: 9400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Fish are vertebrates (i.e. they have a skeleton) so represent a simple yet appropriate model for studying osteoarthritis disease. Zebrafish are an excellent model for these studies for two reasons: the larval stages are virtually transparent, making visualisation of injury dynamics and inflammation possible, and they are easy to use in genetic modification studies. The transparent nature of the larvae makes them uniquely suitable for the visualisation of the cell biology of inflammatory responses within a living host.
We therefore use zebrafish because they offer several technical advantages compared to alternative species

such as mice for most of our experiments.
We will only use mice for a specific surgical procedure that mimics osteoarthritis in human to understand the role of identified proteins in the development of the disease and to validate the mammalian relevance of our findings.
This model is proved to be an extremely valuable preclinical model i in which to carry out initial evaluation of novel potential therapies.

Typically, what will be done to an animal used in your project?

Zebrafish will be used to generate mutant lines of proteins in the ubiquitin system and downstream signalling. These proteins were identified to be modulated by mechanical injury to articular cartilage which is a key risk factor in tissue damage and progression of osteoarthritis. The role of this family of signalling enzymes in activating tissue damage will be tested by deletion in Zebrafish one at a time using gene-editing technology. These procedures involve microinjection techniques to deliver RNA and protein material to induce mutation at 1 cell stage zebrafish embryos (100 procedures over 3 months). We will then characterise the effect of these mutations in zebrafish on the response to inflammatory response induced by tissue injury and on the skeletal system development and function. This involves advanced imaging and histology techniques as well as gene expression and biochemical analysis (500 procedures over 1-2 years).
Success of the use of zebrafish model to mimic the human disease will allow us to use them to help to find new treatments for the human disease. This will usually involve treating the fish with drugs, typically delivered in their diet, and then seeing if drug-treated fish show any improvement compared to fish receiving placebo (200 procedures over 1-2 years). Further analysis of these drug trials will typically involve pathological and biochemical confirmation of the effects of the drug (no additional procedures, over 1 year).

What are the expected impacts and/or adverse effects for the animals during your project?

The types of adverse effects that we expect to see for the larval fish might include alterations to jaw shape and jaw function. We will carefully monitor these fish to ensure that they can feed even if their jaw shapes are abnormal. Occasionally when we are using a new drug or generating a new genetic modification a small number of larval fish may develop adverse effects such as heart oedema or brain malformation, these fish will be killed as soon as these defects are identified.
For adult and ageing fish, we expect the adverse effects to be the onset of joint conditions that resemble human osteoarthritis. These would be likely to cause stiffness in some joints (those of the jaw and the spine) which could in turn lead to reduced swimming performance and reduced speed of the fish. We will monitor the behaviour of the fish daily and any fish that can no longer maintain their position in the water will be killed under terminal anaesthesia. By carefully monitoring fish to ensure that that they do not exceed these end points we expect these studies to be of mild severity.
Procedures to induce osteoarthritis in mouse are of moderate severity. No specific adverse events are expected in relation to this beyond those related to any surgical procedure and therefore any pain associated will be controlled with appropriate analgesics.
Experiments are terminated after 12 weeks following induction of osteoarthritis using a humane endpoint procedure. Knees are then collected and analysed by histology to evaluate the disease progression. Mice will be killed if they show signs of ill health, such as piloerection and hunched posture, inactivity or inappetence that persist and cannot be ameliorated by mild veterinary interventions. In addition, any animal that loses 20% of its body weight when compared to age-matched control will also be killed
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most experiments will use zebrafish.

mild: 95% moderate:5%

Experiments using

mice moderate:100%

What will happen to animals at the end of this project?

- Used in other projects
- Kept alive
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The zebrafish has become widely accepted throughout the world as a particularly useful system to analyse human disease at genetic and molecular levels. The use of Zebrafish as an animal model for this study is essential for in vivo experiments involving targeting genes and generation of knockouts to study the role of signalling enzymes in connective tissue injury models in vivo. These models cannot be mimicked in cell culture or in our model of cartilage injury from pig trotters and is used as a replacement for mammalian models.

Which non-animal alternatives did you consider for use in this project?

In a number of biochemical experiments, a large amount of cartilage tissue for protein analysis studies is required. For that, we use pig trotters (known scientifically as porcine metacarpophalangeal joints) obtained from local abattoirs as a source of articular cartilage and a replacement of using animal models.
Additionally we use ex-vivo studies of articular cartilage models to inform on significance or proteins sets to be targeted in vivo or on the efficiency of chemical compounds and inhibitors.
Furthermore, we established a computational approach to predict the upstream the proteins acting as regulators in ubiquitin system based on proteomics data obtained from ex-vivo studies. This approach reduced significantly the number of animals needed for this study.

Why were they not suitable?

These approaches significantly helped to provide more concise information on the proteins needed to be targeted in vivo but they cannot mimic the actual cellular response occurs in the animal to this challenge. The joint is a complex 3-dimensional structure containing many different cell types and which is subject to frequent movement, as such there are very few non-animal models that exist and none that model all aspects of joint cell behaviour. We therefore need animals to understand what happens to the different cell types during common human joint disorders such as osteoarthritis.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

For those experiments that require animals we perform power calculations to define the minimum numbers required to achieve defined levels of statistical significance and we consult with statisticians and other biologists when planning experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

In this project the advancement of 3Rs is highly implemented in the proposed research. In a number of biochemical experiments, a large amount of cartilage tissue for protein analysis studies is required. For that, we use pig trotters or forefeet (known scientifically as porcine metacarpophalangeal joints) obtained from local abattoirs as a source of articular cartilage and a replacement of using animal models. This also helped significantly to reduce the animal numbers used in this study.
Almost all proposed fish experiments are performed on larvae before the onset of independent feeding– these larvae are not considered protected by the Animals (Scientific Procedures) Act. Procedures for making mutants in zebrafish are mild severity. Genotyping of Adult fish is done on anesthetised fish (small tail biopsy which regrows). To minimise animal use, once experiments have been performed, sperm will be frozen (for future IVF) and stocks will not be maintained.

Mammalian murine models are proposed to test the role of identified enzymes in well-established mammalian models of osteoarthritis. This is essential to investigate the disease relevance of the identified enzymes. Animal usage is minimised by proper power calculations based on previous studies and by having experienced researchers and technicians carry out surgery and histology, enabling us to reduce animal usage and maximise comparison between studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Most of proposed fish experiments are performed on larvae before the onset of independent feeding– these larvae are not considered protected by the Animals (Scientific Procedures) Act. Based on ex-vivo work and proteomics analysis, we have established a computational model to inform on the number of targets employ prediction model to inform on proteins that will be targeted in vivo. Procedures for making mutants in zebrafish are mild severity. Genotyping of Adult fish is done on anaesthetised fish (small tail biopsy which regrows). To minimise animal use, once experiments have been performed, sperm will be frozen (for future IVF) and stocks will not be maintained.
Pilot studies are performed initially to inform on the optimum number of animals needed per experiment to see a significant effect. Spare larvae are used for other experiments or shared with other members of the laboratory for reasons of efficiency.

Mammalian murine models are proposed to test the role of identified enzymes in well-established mammalian models of osteoarthritis. This is essential to investigate the disease relevance of the identified enzymes. Animal usage is minimised by proper power calculations based on previous studies and by having experienced researchers and technicians carry out surgery and histology, enabling us to reduce animal usage and maximise comparison between studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use zebrafish in most of the experiments proposed in this application as a refinement over using mice for processes that are conserved. Zebrafish is the model of the lowest neurophysiological sensitivity in which the role of signalling enzymes in injury responses can be systematically analysed using genetic methods. Compared to mouse models, the adoption of zebrafish is having a significant effect on 3Rs and animal welfare, either by replacing mammalian models, or by informing experimental design in murine systems.

Zebrafish are the only experimental system in which proposed experiments can be performed. In vivo systems are required to model complex multi-cellular processes such as inflammation and injury dynamics. Zebrafish larvae remain the model of the lowest neurophysiological sensitivity. All the direct experiments proposed are performed on animals before the onset of independent feeding (before 5 days of age), not protected under the Animals (Scientific Procedures) Act. Generation of transgenic lines and mutants requires manipulated fish to grow to adulthood, which although considered a regulated procedure, is
“sub-threshold” in terms of the impact on the animal itself. Compared to mouse models, the adoption of zebrafish is having a significant effect on 3Rs and animal welfare, either by replacing mammalian models, or by informing experimental design in murine systems.
Mammalian murine models are proposed to test the role of identified ubiquitin enzymes in well-established mammalian models of osteoarthritis. This is essential to investigate the disease relevance of the identified enzymes. Animal usage is minimised by proper power calculations based on previous

studies and by having experienced researchers and technicians carry out surgery and histology, enabling us to reduce animal usage and maximise comparison between studies.

Why can't you use animals that are less sentient?

We have chosen the zebrafish as most refined animal model available (as worms and flies do not have a skeleton). By using the translucent zebrafish in which we express fluorescent proteins in cells of interest we can non-invasively watch many cellular processes in the skeleton, allowing us to collect dynamic data with minimal surgical intervention.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimise suffering and discomfort the animals will be monitored daily and when there is any concern advice will be sought from the named veterinary surgeon and/or the Named Animal Care Welfare Officer and appropriate action taken.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Our laboratory is fully conversant with ARRIVE guidelines for the planning and reporting of animal experiments and fully compliant with best practice recommendations.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our group will stay informed about advances in the 3Rs through a number of channels of communications including animal users meetings, NC3Rs symposia and updates from husbandry and welfare conferences, collaborations and through direct advice and consultation with NTCO, NVS and NACWOs.

We aim to implement these advances in our work as appropriate.

Home Office

NON-TECHNICAL SUMMARY

177. Targeting therapeutics to sites of disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Targeted delivery, Gene Therapy, Immobilised molecules, Nanovehicles, Triggered release

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Whilst there has been tremendous progress in drug development and disease treatment in the last half century it is evident that most drugs are delivered systemically (oral or injection) , but often their action is only required at specific disease sites. Delivering, targeting and retaining molecules at sites of disease or developing approaches to control or trigger drug activity are the main themes of the work in this project licence application. We will explore different approaches to target treatments to sites of disease with the aim of increasing their efficacy whilst reducing side effects. The technologies we will be exploring range from genetic elements and protein engineering through to miniature vehicles. Many of the technologies are as a result of interdisciplinary collaboration between life sciences and materials engineering so we will be looking to develop novel delivery methods through the work of this licence. The primary target for our therapeutics is inflammation in joints. Many

therapeutics currently utilised clinically have significant ‘off target’ effects such as systemic immunosuppression which leaves patients vulnerable to opportunistic infections. We will be keen to determine if we can achieve long-term targeted effects from a single delivery; if we can control therapeutic delivery and if we can mirror delivery of therapeutics to the course of disease activity. If we can achieve these outcomes we are confident that this will aid in the development of new specific treatments for diseases.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Targeted therapeutics have the potential to be more efficacious because they are not distributed around the whole body but are selectively delivered to the disease site. Targeting will also reduce ‘off target’ side effects. Better targeting of treatment will mean that patients will be more effectively treated, have fewer complications of treatment due to side effects which will ultimately lead to better treatment outcomes for patients and clinicians.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

All the work proposed in this license is focused on the use of mice. Experiments will be designed with the aim of using the minimum number of animals that will determine if significant effects are achieved. All experiments will be informed by statistical power analysis and we will seek the advice of a resident statistician when necessary. I envisage that 1500 animals will be used over the 5 year lifetime of the license.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Inflammation in joints causes pain, swelling and discomfort which can restrict movement to some degree. In this licence acute inflammation will be used which is short-term and resolves within a matter of days and has a severity limit of moderate. If suffering exceeds expected levels animals will be provided with analgesia and if their welfare does not improve animals will be humanely killed by an appropriate method.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

In programme of work described in this licence is aimed at achieving better delivery of drugs at disease sites through molecule design and through the use of drug carriers. Initial experiments have been performed with the molecules and drug carriers in experiments where we have tried to simulate aspects the disease environment. When the performance of molecules and carriers is effective and optimised in these systems they are ready for evaluation in an animal model. Mouse inflammation models have the advantage that we can monitor interactions of our molecules and carriers with the immune system and complex living structures which cannot currently be replicated in our laboratory based experiments. Should suitable alternatives to animals become available during the course of the proposed work then we will evaluate their application in these studies.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Throughout the work of this project animal numbers will be kept to a minimum whilst ensuring that data obtained is rigorous and reproducible. This will be achieved by using a stop/go approach in experiments in disease models that are very reproducible which will mean that group sizes will be smaller and overall animal usage will be less. By this approach we will get an early indication that treatments are very effective or are ineffective following the use of small animal groups which will indicate if experiments should continue (go) or end (stop). Power analysis will be used in experimental design in order to ensure that group sizes are powered sufficiently to detect statistically meaningful effects.

State of the art imaging modalities will be utilised to enable monitoring at multiple time points in the same animal which not only overcomes issues with inter-animal variability but also greatly reduces animal usage. Imaging in anaesthetised animals can be used to demonstrate targeting of therapeutic or drug delivery particles in the whole animal at multiple time points. Where a single group of mice can be used for the whole experiment using imaging now, multiple groups of mice would have been used for each timepoint in the past.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The mouse models provide the opportunity to examine our drug delivery systems in inflammation models that reflect aspects of human disease. The focus of the work in this license is joint inflammation so the outcomes of the work are of most relevance to diseases of this structure such as rheumatoid arthritis. There are many anatomical and functional similarities in the joint structure of rodent and human joints and the disease models listed in this license replicate changes that are seen in human disease. The models planned cause inflammation in a single paw and animals can readily move around their cage which is more refined than other models where inflammation develops in all paws. During the inflammation animals will be routinely measured and clinical signs will be monitored so that any intervention (such as analgesia) or humane end point can be employed promptly.

Home Office

NON-TECHNICAL SUMMARY

178. Tau protein, glia and synapse damage in neurodegenerative diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Alzheimer's disease, Dementia, Therapy, Understanding disease

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We are interested in how changes to proteins disrupts communication between brain cells to cause dementia and other features of neurodegenerative diseases. We will use models of neurodegenerative disease to study alterations in protein structure, function, localisation and spread throughout the brain, on nerve cell health. We are also interested in how these processes are affected by the activation of immune cells, in the brain.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Alzheimer's disease and related neurodegenerative diseases that feature abnormal tau are the leading causes of dementia. The prevalence of dementia is increasing because age is the biggest risk factor and the global population is living longer. There are no therapies to slow or prevent dementing diseases, and this is in part due to an incomplete understanding of changes in the brain in dementia. There is emerging evidence that abnormal forms of tau protein act as an "executioner" to damage nerve cells and their connections (synapses), to cause dementia. Our work will build on these studies to better understand how tau changes in healthy ageing and in models of neurodegenerative dementing diseases. We hope that this work will identify new routes for treatment.

What outputs do you think you will see at the end of this project?

We expect this project to inform our understanding of how modifications in tau, occur in response to changes in nerve cells and other brain cell types that mediate inflammation. We seek to understand how these alterations affect disease progression and the communication between nerve cells and so underlie the clinical features of Alzheimer’s disease and related neurodegenerative disorders. The project will produce data that we will make openly available to scientists and other interested parties, and that will be included in open access publications. We will also present our work in other academic institutions, scientific conferences and in public engagement events.

Who or what will benefit from these outputs, and how?

The most immediate impact of our outputs will be on the scientific community, who will be able to use our results to expand or direct their own research. The subject of our research is a priority area and there are many groups around the world investigating related topics. It is hoped that in the long term our findings will be used to help devise or support the development of new therapeutic agents for Alzheimer’s disease and dementia. Importantly, our data might provide scientific “proof of concept” in support of clinical trials, as we have done before.

How will you look to maximise the outputs of this work?

We will maximise the outputs of our work by presenting our findings to a range of groups, including members of the public, healthcare providers and other key stakeholders at public engagement events or in funder publications. We will ensure that there is open access to our publications and data collections. We have previously published methods for some of the key protocols that we will use in this project license, and we will do so again for updated or new methods. This is important to allow others to reproduce our findings in their own labs and using their own reagents and models. We have trained researchers from pharmaceutical companies

and other laboratories in our methods to promote their uptake, and we will continue to do so. We will also endeavour to publish unsuccessful studies to inform the research community. This is important since reporting unsuccessful experiments will prevent other labs from using animals unnecessarily, wasting time and other resources to perform experiments that we have already shown are unsuccessful. Ruling out particular ideas will focus future research efforts into experiments with more chance of success, which should advance this field of research more rapidly.

Species and numbers of animals expected to be used

- Mice: 6000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice in this project since these are the least sentient species that allow us to produce meaningful results. We must conduct our research in vivo since we are examining changes in the brain that occur as a result of aging and as a consequence of Alzheimer's disease development. Less sentient animals do not have conservation of the brain structures that are most affected in Alzheimer's disease. In addition, the processes we are studying involve nerve cells and non-neuronal cells in the brain, and spread of abnormal proteins as a result of brain activity, and these aspects are very difficult to recapitulate without using an intact animal. However, some aspects of our project can be studied using a combination of primary neural cell cultures and we do this when it is appropriate to answer specific questions, with results related to those gained in experiments using human cells. We also culture brain slices that are prepared from postnatal animals for many of our experiments, since these can be aged in in vitro and they retain brain anatomy and connectivity across the slice.
We use pregnant mice, and embryonic mice to generate primary neurons. Postnatal mice are used to make long-term slice cultures. Protocols for these methods are well established and have been shown by us and others to be optimal at these developmental stages. In addition, aging brain slices in a dish negates the requirement to age mice until such time as they display detrimental age- or neurodegenerative -disease associated changes. We also use adult and aged mice to examine key age- and neurodegenerative disease-associated changes for comparison with postmortem human brain and validation of our culture models.
Typically, what will be done to an animal used in your project?

The work planned in this project falls into three main categories:

1. Normal and genetically modified mice will be aged typically to a maximum of 2 years of age. Brain and other tissues from these animals will be collected for examination of age- and disease-related changes in cell types, proteins, structure and markers of brain function.

2. Young mice may be used to provide brain tissue for primary neural cell and long term brain slice cultures.

3. Some normal and genetically modified mice may be treated with substances that affect ageing or neurodegenerative disease progression for up to 2 months, either as juveniles, adults or aged mice.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals on this project that are maintained out to old age may develop various age-related issues that are common to mice, such as benign tumours or hair loss, and in some cases sore patches on the skin. If these effects are only mild, and cause minimal distress, the animal may be maintained for several months, however, if any of these issues are thought to cause significant pain, discomfort or distress, the animal will be culled.

Animals modelling dementia may have learning and memory problems, that worsen throughout their life. However, these are not expected to have any major welfare issues for the affected animals, and a small number of animals will be kept for a maximum of two years as these memory problems develop. Some of these mice may also develop problems with mobility and motor functions. These are not expected to be accompanied by any pain, and in most cases, will not affect the mouse's ability to move around their home cage, feed, drink or groom.

A small number of animals may be treated by injection or by addition of substances to their food or drinking water to treat or exacerbate or disease. The injection is not expected to cause anything more than minimal discomfort, and those receiving drugs would in the most part be expected to improve. Treatments are short-term and should animals develop any sustained adverse effects, the study would be halted and animal treated accordingly.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most of the experimental work planned in this project will result in mild and transient harm to mice. A small proportion of work will cause moderate harm, as a result of ageing mice until they show disease phenotypes, or as a result of treatment with agents that may accelerate disease to help us understand the underlying biological causes. So, we estimate that 50% of animals will experience only mild harm and the remaining 50% will experience moderate harm.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project aims to increase our understanding of the molecular events that occur in human neurodegenerative diseases. These diseases worsen as time passes. We seek to better understand the changes that occur in the brain as a result of ageing and worsening of neurodegenerative disease over time. Therefore, we must study an animal with a brain structure that is similar to humans, that has similar cells, cell connections and proteins, and in which disease worsens in a similar way to human disease. Both the complex circuitry of the brain and the process of aging are challenging to study outside of a living animal, hence using animals is generally considered as the most appropriate and sensitive way to address these questions.

Which non-animal alternatives did you consider for use in this project?

Human cell lines, human neurons and non-neuronal cells, and human postmortem brain.

Why were they not suitable?

It is essential to the aims of this project that we use a system in which we can study and manipulate brain changes that occur with aging and as neurodegenerative disease worsens over time. These complex changes cannot be replicated in cell lines, which do not grow or age like brain cells. Most protocols for establishing human brain cell cultures also involve steps that mean that the cells are newborn, therefore limiting their utility for ageing studies unless they are aged for years which is technically challenging. We do, however, use human nerve cells and other brain cells for aspects of our work, as appropriate. We also make use of postmortem human brain, which allows us to take snapshots of processes at different disease stages, but since these tissues are obtained from single individuals at specific disease stages, changes in each brain cannot be followed or the tissues manipulated.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The work outlined in this project will allow several projects that are aligned with the objectives of this study. We have calculated carefully the number of animals required to address the aims of each experiment and produce robust data, without using more animals than absolutely needed. Our previous experience, combined with published information and formal calculations, allows us to make informed judgements about how many mice, or primary cultures of mouse cells, are needed for routine experiments. For small scale pilot studies, numbers of animals will be kept as small as possible, and will be based on our and others prior experience of similar studies. In the case of follow-up studies for this new work, current number estimates are based on those typically required for similar experiments, but specific calculations will be conducted for each experiment to determine a sufficient number of animals before the experiment begins. We also take into account how many mice are required to maintain healthy colonies of each line.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

During the design phase of each experiment, where information was available, appropriate searches were conducted on each mouse line of interest to ensure we had access to all relevant information regarding expected outcomes in these animals. This knowledge, combined with our own extensive experience with these lines drove the calculations that informed our determination of animal numbers for each experiment. Extra consideration was made for a number of variables, including sex, age and background strain. The statistical approaches to be used at the end of the study formed a major part of the experimental design process, ensuring that all experimental approaches are robust.
The experimental design was also discussed with other researchers familiar with these kinds of studies, to further validate the design, and to ensure that we had included adequate control groups to each experiment, such that all data obtained will be valid.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have replaced much of the work that would previously have been conducted in mice, with studies using long term brain slice cultures. These methods allow us to examine several parameters (age, treatment) in tissue from a single animal. We also ensure that tissue collected from these cultures or from living mice is processed and stored to ensure that it can be used for multiple types of analysis (e.g. biochemical methods and pathological characterisation). All organs of interest will be harvested from each animal, and experiments will be designed to allow multiple follow ups in the tissue from each animal, thus minimising the total number of animals required. Thus, we aim to maximise the amount of data that we can obtain from our experiments.
In many cases, small scale pilot studies will be conducted prior to each full-scale study, with follow up analyses to ensure both that the objective is a valid one, and also that experimental design is optimised to use the minimal number of required animals. This will ensure that unnecessary or poorly planned large-scale experiments do not take place.
As far as possible, breeding strategies will be designed so that all animals from a mating are used for an experiment, and for general maintenance of a line, animal breeding will be monitored and controlled to ensure that we obtain sufficient mice to maintain the line, while minimizing the birth of mice that are not required.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use mice since they are the lowest animal species that is sufficiently similar to humans to allow us to answer our research questions. We are interested in the spread of abnormal proteins in neurodegenerative diseases, and how brain cells other than nerve cells affect the connections between neurons to facilitate disease progression. Mice are the lowest mammalian species that show similar brain anatomy and regional connectivity that is similar to humans, that have similar non-neuronal and neuronal cells present and that allow us to study the effects of ageing.
We will use genetically altered mice that are considered to be the most appropriate and relevant for each experiment. These include mice in which:

1. Genes have been removed to enable us to understand their function for health and disease.

2. Human gene mutations have been introduced into mouse genes to allow us to determine how the mutation causes neurodegenerative disease.

3. An entire human gene has been introduced at low levels to allow us to investigate how these genes cause neurodegeneration as mice age.

Why can’t you use animals that are less sentient?

Abnormalities in human neurodegenerative diseases such as Alzheimer’s disease arise in specific brain regions and spread to other areas as the disease progresses. Each brain region possesses a different complement of cell types and connections that is considered key to this pattern of spread. In order to investigate disease progression in animals, it is essential that we use a model in which brain regions and their anatomical connections are similar to those in humans. Mice are the least sentient species of animal that meet these criteria. In particular, mice have a cerebral cortex and hippocampus with similar anatomy and functions to these areas in humans. These regions are critical for learning, memory and executive cognition that are affected in Alzheimer’s disease and related disorders. In addition, some of the key molecular pathways that are important in Alzheimer’s disease are not present in less sentient species such as fruit flies, worms or fish. These organisms would have to be further manipulated to make them suitable for our investigations. They also have a short life span, limiting their utility for studying age-related neurodegenerative diseases.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have refined our experiments to selectively use animal models that develop only mild-moderate features of human neurodegenerative diseases. We do not use animals that show the harmful features of terminal disease stages. We generally use the least adverse methods that are compatible with the aims of the study. For example, we preferentially study young rather than aged mice in which there are changes in brain chemistry, but no psychological or outward physical signs of disease. When we treat animals, dosing regimes will be designed to minimise stress and suffering to the animal. Animals will be handled prior to the onset of the experiment, to acclimatize them to this handling prior to the administration of any substance. Animals receiving substances will be particularly closely monitored during the initial treatment phase, to check for any unexpected issues that might arise.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow ARRIVE (most recently ARRIVE 2) guidelines when planning and conducting our research. We always include ARRIVE checklists with our publications.
We also take into account, and act accordingly, on recommendations and guidance provided by our NC3Rs liaison, and dedicated animal management, support and care teams.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We have previously received funding from NC3Rs, an organisation committed to furthering 3Rs principles, to support our work and have attended many seminars/symposia and roadshows organised by their team so that we remain up to date with advances in this area. We receive regular communications from NC3Rs directly, and through our Institution.. We will implement, with advice from our vet and other trained and experienced staff, any recommendations that are in-line with the work outlined in this license and provided that any such change is not expected to have an impact on the animals such that it may alter research outcomes.

Home Office

NON-TECHNICAL SUMMARY

179. Testing advanced contrast agents to improve MRI diagnostics.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

MRI, hyperpolarisation, oncology

Animal types	Life stages
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to improve the quality of the pictures taken with magnetic resonance imaging (MRI); towards delivering better diagnostics in cancer. It uses novel chemically derived, injectable, 'contrast agents' to achieve this.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The direct monitoring of cell based activity in living animals will enhance our ability to treat disease and monitor subsequent progression. Currently the standard anatomical pictures that MRI produces are not sensitive enough to see the necessary subtle cellular changes.
If the pictures MRI takes could be improved so that the cellular development of disease could be seen, then it would be become possible to make earlier diagnosis and view the response to treatment.

What outputs do you think you will see at the end of this project?

We aim to produce evidence to show that novel MRI-based contrast agents permit the visualisation of cellular changes in health and disease. Pictures will be taken to map changes in cancer and blood flow.
Data from individual studies will be shared widely through journals, scientific conferences and meetings with clinical bodies to ensure the wider benefits are realised.
The intellectual property (IP) backing this project will be used to support the commercial developments needed to make this approach generally available.

Who or what will benefit from these outputs, and how?

Short-term (0-5 year) benefits:
We will produce a range of safe contrast agents for use in pre-clinical MRI to examine disease. The methods we use to take MRI pictures will be shared to help others. We expect to generate IP that will be of benefit to the UK.
Medium-term (5 year) benefits:
The resulting portfolio of information will support applications to the Medicines and Healthcare products Regulatory Agency for approval to use our methods in the clinic.
Long-term (6 year+) benefits:
Improved MRI picture quality will allow clinicians to map a range of bio-chemical functions within the cells of the human body. This will help them in diagnosing and tracking tumours, heart disease and a range of brain diseases (such as Alzheimer's and Parkinson's). Our methods offer quicker measurements, at low cost, thereby allowing doctors to offer such investigations to a growing number of patients whilst simultaneously improving outcomes. We expect these methods to help reduce the number of animal experiments needed in pre-clinical MRI.

How will you look to maximise the outputs of this work?

We are already collaborating with leading clinicians and researchers to identify suitable agents to use in the first instance. As the project develops we will seek to collaborate more widely as our technology is further recognized for its ability to transform the diagnostic use of both pre-clinical and clinical MRI. Beyond peer-reviewed publications, we expect this to be done through a dedicated website as a forum for targeted webinars.

Species and numbers of animals expected to be used

- Rats: 220

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult rats are a well-defined model for imaging research. The MRI equipment we are using is specifically designed for imaging these animals.

Typically, what will be done to an animal used in your project?

All animals will be anaesthetised, given pain killers and a contrast agent. They will then be examined by MRI over a period not exceeding 3 hrs. Respiration rate, heart rate and blood pressure will be monitored throughout. The animals will not recover after the MRI step. 100 of the rats used in this work will reflect a cancer model to test agent pooling and detection.
Data from all animals used can also inform us about perfusion (how fast agents reach particular organs/tumour sites).

What are the expected impacts and/or adverse effects for the animals during your project?

The animals used should not experience pain or discomfort, with pain relief and anesthics administered as appropriate. Any animal found to be suffering, following appropriate care, will be immediately and humanely killed.
Some of our rats will be used to grow subcutaneous tumours. Tumour size will not be large enough to cause discomfort. They are all humanely killed at the end of the experiment, and before they undergo any suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Our MRI experiments will be done under terminal anaesthesia where the animals will not be allowed to wake up,

so-called non-recovery experiments (220 rats).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Where possible, we make extensive use of alternative experimental resources, including computer simulations and cell culture models. It is impossible to accurately mimic the physiological conditions required to validate our novel MRI contrast agents through such methods. Thus, it is essential to use an experimental model such as the mouse/rat if these approaches are subsequently to be used for the diagnosis of disease in both animals and humans.

Which non-animal alternatives did you consider for use in this project?

Our contrast agents have already been tested widely in non-animal experiments. This has included simple test tube based imaging through to in-vitro models of blood-brain-barrier permeability.

Why were they not suitable?

While these studies have demonstrated that we can dramatically improve MRI picture quality, they do not account for the effects of physiology in living animals. We need to understand the effects of diluting our agents in a circulating blood system down to the capillary level. In addition we need to ensure that there is no significant change in blood pressure, breathing rate, heart rate and temperature as a result of agent injection. We need to understand whether transport times within the body from the injection location to the disease site are suitable.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

All experiments are designed to use the minimum number of animals while still producing scientifically and statistically significant results. Animal numbers were estimated based on previous experience, discussions with the Medicines and Healthcare Products Regulatory Agency and published data.
The use of medical imaging allows the same animals to be studied over differing time points in life and thus increases data obtained whilst drastically decreasing animal numbers as without imaging animals would be killed at each time point to gain necessary data.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

All experiments are designed to use the minimum number of animals while still producing scientifically and statistically significant results.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Each new contrast agent will be tested on cells in a test tube to make sure they are safe before any invivo injection is done.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats are used because they age similarly to man. Furthermore cancer can be induced as in man.
The animals used in this work will include healthy rats and rats with tumours. By selecting existing models we can ensure that we are fully aware of any potential health concerns.
The studies will be completed under terminal anaesthesia from which the animal will not wake. Pain killers will be applied in conjunction with anaesthetics to make sure the animals feel no pain.
Imaging quality is improved/refined by ensuring that animals are kept still during the imaging process by anaesthesia.

Why can’t you use animals that are less sentient?

The rat models we propose to use mimic the corresponding disease in humans.
The physical limitations of the MRI system mean that work will start on rats as less sentient fish would be difficult to work with in terms of both required size, injection of a contrast agent and development of a water circulation system to keep the fish in a good physiological state.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Pain killers will be applied to ensure the animals feel no pain. Top-up doses of anaesthetic will be delivered as required to prevent animals waking up during experiments. If any procedural complications do arise advice will be sought immediately. During the programme we will ensure we remain fully up-to date with relevant new procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Injection volumes based on Recommended Dose Volumes for Common Laboratory Animals document
- IQ 3Rs Leadership Group (Jun 2016): https://iqconsortium.org/
PREPARE guidelines for experimental planning: Smith, AJ, Clutton, RE, Lilley, E, Hansen KEAa, Brattelid, T. (2018): PREPARE: Guidelines for planning animal research and testing. Laboratory Animals, 52(2): 135-141.

The Experimental Design Assistant (EDA): https://www.nc3rs.org.uk/experimental-design-assistant-eda

LASA for aseptic practice: http://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The local scientific community operate forums twice a year to pass on updates and support for the 3R's more generally.
We will keep up to date on the latest news regarding the 3Rs via the national centre for the replacement, refinement and reduction of animals in research via their dedicated website, blogs and social media accounts.

Home Office

NON-TECHNICAL SUMMARY

180.Testing and Assessing FMD Vaccines

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Cattle	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The aim of this project is to assess the immunogenicity and efficacy of vaccines to foot-and-mouth disease virus.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Through testing vaccines in our facility we will be better equipped to deal with any future incursions into the UK. Internationally, we will be able to advise countries which vaccines are more efficacious for the strain they may be dealing with and whether there is any cross-protection to other strains that may be circulating in neighbouring regions.

What outputs do you think you will see at the end of this project?

This programme will contribute to national, European and global control of FMD. Vaccination is a fundamental part of FMD control measures and there is a need to confirm the efficacy of existing vaccines for field use. There is a regular need to identify new vaccine candidates to ensure that there are suitable vaccines for emerging FMDV strains. Once identified, these require additional testing to produce safe, potent, efficacious and stable vaccines. This work will have a global impact on animal health as it will help with international control and eradication of this economically devastating disease of cloven hoofed domestic and wild animals. Further vaccination with new adjuvants and constructs or manipulated capsids may increase the duration of immunity to FMD and the development of DIVA or marker vaccines for FMD will protect target species and act as efficient discriminatory tools to support serological surveillance and confirmation of disease free status.

Who or what will benefit from these outputs, and how?

A reliable supply of safe, potent and effective vaccines is essential for the maintenance of animal health and the successful operation of animal health programmes. Immunisation of animals with high quality vaccines is the primary means of control for many animal diseases including FMD.
The information gathered from these studies will ensure that the organisation is in a strong position to offer national and international advice on FMD. We will also have the ability to supply materials to research and diagnostic groups within the organisation to enhance our research activities into the infectious processes and immune responses associated with FMDV in the target species and our diagnostic capabilities.
How will you look to maximise the outputs of this work?

The approach to the data generated from non regulatory studies carried out under this PPL, whether successful or unsuccessful, will be published in peer reviewed journals to enable the immune responses to FMDV infection and or vaccination to be disseminated to the community.
Regulatory data remains confidential however this will be utilised to enable market authorisation for vaccines to be obtained, which has an obvious direct maximisation of output as this will lead to increased vaccine uptake being available across the globe.

Species and numbers of animals expected to be used

- Cattle: 420

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

These animals and their ages are being used as these are mandated in the international OIE and EU standards for assessing Foot-and-mouth disease vaccines.

Typically, what will be done to an animal used in your project?

Animals will have blood samples taken at periodic time points throughout the course of the study, typically before vaccination and after vaccination. Animals may then have a vaccination. If the immunity of vaccinated animals is also being tested, animals will be injected with foot-and-mouth disease virus and may have blood and swab samples collected after challenge. A typical batch test of vaccine will take 21 days, and a potency test (assessing protective ability of the vaccine) will typically take 29 days. **What are the expected impacts and/or adverse effects for the animals during your project?**

Animals which are only vaccinated with vaccine are expected to only experience mild severity. Animals which are challenged with live virus are expected to experience moderate severity.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All animals which are only vaccinated with vaccine are expected to only experience mild severity. All animals which are challenged with live virus are expected to experience moderate severity. it is expected that around 70% of animals on this licence will experience moderate, and 30% mild severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The testing of vaccines requires the host species to be used under regulatory and licencing authorities. However, prior to animal testing, vaccine strains are screened using cell culture techniques to help match them to field viruses against which protection is sought and to check that they have growth and stability characteristics

suitable for vaccine manufacture and storage.

Research is ongoing to develop improved methods to evaluate vaccine performance in the field, reducing reliance on the use of experimental animals; some of the work from this project will generate data that can be used in models already developed to assist with their validation.

Which non-animal alternatives did you consider for use in this project?

It is not possible to assess vaccine efficacy and immunogenicity for regulatory purposes without using animals as the set international procedures stipulate the test. Titration of cattle adapted virus is now undertaken in vitro, animals are not used.

Why were they not suitable?

The regulatory tests could not be achieved without the use of animals as the law stipulates the test required.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This is based on:
2 Potency tests conducted per year, (17 animals per test) for 5 years = 170
8 batch tests per year (5 animals per test) for 5 years = 200
3 generalisation studies per year (2 animals per test) = 30
2 production studies per year (2 animals per test) = 20

Total = 420

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

These are the minimum number of animals used according to the OIE manual and EU Pharmacopeia.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Whilst the numbers of animals used in these studies is fixed by regulatory requirements, post-mortem tissues will be shared with other researchers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

For safety and potency testing of these veterinary vaccines the target species for the products have to be used. To minimise suffering, we will ensure that the vaccines have been formulated in such a way as to maximise its chances of being efficacious before it is put into cattle, or that preliminary studies have been carried out to demonstrate immunogenicity before challenge. We can also use medicines under direction of the on call veterinary surgeon to reduce clinical signs.
When quantifying the virus in animals, all animals will be heavily sedated to reduce stress, and also allow more precise administration of virus to be given into the insensible target area of skin, thus reducing pain. Pain relief may be given before and during infection to reduce some of the clinical signs.
We have very strict humane end points and very well trained staff to prevent unnecessary suffering.

Why can’t you use animals that are less sentient?

international regulatory requirements stipulate cattle must be used to assess foot-and-mouth disease vaccines. This ensures that there is maximum confidence that the vaccine will protect cattle when they are vaccinated with it on farms around the world form FMD.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be housed together with bedding and other items of enrichment. Highly trained animal technicians will monitor these animals throughout the day, ensuring they are comfortable and to maximise their welfare status. We have 24/7 CCTV surveillance which can be used to monitor the animals behaviour over time.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Adherence to the ARRIVE guidelines for reporting these studies, as well as to the FELASA guidelines for large animal health monitoring to help ensure the most robust health assurance for animals used in this study.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through continued CPD and frequent review of the CAAT (Centre for Alternatives to Animal Testing) I will keep informed about advances in the 3Rs. Included in CPD will be annual attendance at national lab animal science conferences as well as naturally reviewing the current literature surrounding infectious disease research, as well as attending relevant FMD conferences where updates and best practices are discussed.

Home Office

NON-TECHNICAL SUMMARY

181. Testing the efficacy of new vaccines against Clostridium difficile in two clinically relevant animal models

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

vaccines, mucosal, Clostridium difficile, infectious disease

Animal types	Life stages
Hamsters	adult
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To test the immunogenicity and protective efficacy of different vaccines given by different routes against Clostridium difficile infection (CDI) in two well established animal models.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

C. difficile is the leading cause of hospital-acquired infection worldwide, responsible for around 500,000 cases a year in the US alone, claiming the lives of around 30,000 people and costing $5Bn. In England and Wales, around 13,000 cases of CDI are reported each year costing the NHS over £120M (Public Health England, 2017). It is typically antibiotic-induced yet ironically the mainstay of therapy is Vancomycin or Metronidazole which, when taken, result in up to 35% of patients succumbing to a second infection and up to 60% of these, suffering a third infection.

There is no effective vaccine against CDI. After the Centers for Disease Control and Prevention (CDC) announced in 2013 that CDI is an urgent public health threat, three vaccines entered clinical trials, fast tracked by the FDA. All three vaccines are administered intra-muscularly and are based on inactivated forms of the 2 secreted C. difficile toxins, TcdA and TcdB. By the end of 2017, a Phase III trial was terminated. This vaccine generated high titres of systemic toxin-neutralising antibodies but did not prevent colonisation by C. difficile and thus failed to confer local protection in the gut. Given the similarity of the two vaccines remaining in trials to that of the failed trial, there are concerns that both will fail. Clearly a new vaccine is needed that specifically targets the gut to generate local antibody protection against CDI.

Our vaccines are designed for oral or nasal delivery to target the gut. The work proposed will generate proof of concept data with licensure of our technologies and prototypes to Pharma who can ultimately take our products to market. To this end, a Confidentiality Disclosure Agreement (CDA) for a collaboration with world-leading Pharma on vaccines has recently been signed.

What outputs do you think you will see at the end of this project?

New information

At the end of this project, we will have determined which type of vaccine and which delivery route (mouth or nose) is the most suitable to get protection against infection by Clostridium difficile in 2 clinically relevant rodent models.

Products

We would expect to have filed patents on some of our vaccine platform technologies and licensed these out to Pharma. We would also like to spin out a company based on the most successful technology which we will then apply to other infectious diseases.

Publications

We plan to publish our work in peer-reviewed journals following patent filing.

Presentation at conferences

We will present our findings at conferences locally and internationally.

Who or what will benefit from these outputs, and how?

In 2-3 years, completion of proof of concept data for our vaccines including immunogenicity and protective efficacy in hamsters and mice, and for some of these vaccines, identification of the most suitable delivery route.
In 2-3 years, patent filing of at least one vaccine platform with potential to spin out a company on vaccine platform technologies or licensure to Pharma.
After 3 years, dissemination of our findings in publications and at conferences.
In collaboration with Pharma, we expect to see one or more of our vaccines transition from our preclinical testing to human trials within 3-5 years.
Beyond this project, we will apply our platforms to higher priority pathogens such as ETEC and Shigella.

How will you look to maximise the outputs of this work?

I will apply for substantive translational funding for example, REDACTED. This will be aided by my collaboration with the world Pharma leaders in vaccine development. They have the infrastructure and finances to manufacture our products in accordance with GMP standards and perform clinical trials.

Once our platform technologies are patented, our data will be disseminated at conferences including the REDACTED, and through publications.

Species and numbers of animals expected to be used

- Hamsters: 784
- Mice: 784

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

It is important to test our vaccines in more than one animal model to acquire sufficient confidence in their efficacy. Hamsters (Golden Syrian) and mice (C57Bl6 and Balb/c) are the lowest vertebrates which display the same CDI disease symptoms as those observed in humans. The hamster is particularly susceptible to C. difficile but both rodents display the full spectrum of clinical symptoms including diarrhoea due to toxin-induced damage of the caecum, analogous to toxic megacolon in humans which manifests as wet tail and weight loss in hamsters, and as loose faeces and weight loss in mice. Within 48 to 72 hours post infection, the humane endpoint is typically reached. Comparing the length of time from infection to the humane endpoint between control and vaccinated groups is a powerful indicator of the success or failure of the vaccine being tested. Young adults of either sex are used for CDI studies, as shown in the literature, as they are capable of mounting

effective antibody responses. Hamsters are typically 10 to 12 weeks old weighing 100-130 grams and mice typically 6-10 weeks weighing 20 - 25 grams.

Typically, what will be done to an animal used in your project?

The procedures to be conducted include;

1. Optional general anaesthetic for animals to be immunised intranasally,

2. Immunisation with vaccines orally or intranasally. Animals will be immunised typically 3 times over 30 days to mount an antibody response. This is standard for C. difficile vaccines. Some animals will be euthanized around day 45, the experimental endpoint for this protocol (Protocol 1). Other animals will go on to Protocol 2 as described in procedures 3 and 4 below;

3. Administration of antibiotics. For hamsters, single dose orally with a wash-out period of up to 5 days before challenge with C. difficile. For mice, antibiotics in drinking water is given for 3 days then normal water for 2 days before challenge with C. difficile.

4. Challenge with C. difficile orally after the antibiotic wash-out, typically day 50. Animals will be closely monitored. Quantitative data will be obtained on length of time to established infection with humane end point assessed using an established monitoring scheme.

5. Vaccinated animals that do not show clinical symptoms up to 2 weeks post challenge will undergo antibiotic administration again to test for persistent protection of the vaccine being tested, against recurrent CDI. Briefly, antibiotic will be given in drinking water for 7 days followed by normal drinking water.

If they do not develop clinical signs after antibiotic administration for the second time, they will be culled 2 weeks after the last day of receiving antibiotics.

6. Once animals reach the humane end point, they will be killing by schedule 1 killing. Animals that show no clinical symptoms 2 weeks post re-administration of antibiotic will also be killing by schedule 1 killing (experimental endpoint). Serum and tissues will be collected for analysis. Total bacterial load in the caecum and histological changes in the caecum and intestinal tissue will be determined.

The duration of Protocol 1 is typically 45 days and the duration of protocol 2 is about 85 days for each experiment.

What are the expected impacts and/or adverse effects for the animals during your project?

1.General anaesthetic
All animals are expected to make a rapid recovery from the anaesthetic.
2.Immunisation studies.
We have not seen any adverse effects of our formulations. The majority of animals would be expected to experience some minor discomfort from administration.
3.Administration of antibiotic.
No adverse effects expected.
4.Challenge studies.
Animals in placebo groups are likely to experience moderate levels of severity. This is because they will develop symptoms of CDI usually 48-72 hours post infection. All animals showing signs of CDI will be monitored closely. The main clinical symptoms covered by the scoring system include diarrhoea which manifests as wet tail and weight loss in hamsters, and as loose faeces and weight loss in mice. Other symptoms monitored are indicative of poor well-being and include hunched posture, stary coat, pilo-erection, reduction in activity and response to stimuli, sunken eyes and unsteady gait.. For each parameter, a score of 0 to 3 is given; 0 for no change, 1 for a minor change, 2 for moderate and 3 for major change from the norm. The scores are then added together and

any animal scoring 15 or above will be humanely killed using a Schedule 1 method. For the weight parameter alone, any animal losing
20% of its starting weight or losing 10% between two concurrent welfare checks will be humanely killed.
Animals in vaccinated groups are likely to experience milder levels of severity because the vaccine is aimed at interfering with the colonisation of C. difficile and preventing the onset of disease symptoms. Those animals not displaying clinical symptoms of CDI up to 2 weeks after challenge with C. difficile will be given antibiotic again to monitor persistent protection of the vaccine. The experimental endpoint is 2 weeks after re-administration of antibiotic.
5.Schedule 1 killing. Animals will be exposed to the effects of the anaesthetic induction at the end of the study (terminal procedure).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

100% of animals in negative control groups (not immunised) are likely to experience moderate levels of severity as they are expected to develop CDI. This equates to one third of the animals (n=8).
Vaccinated animals are likely to experience milder levels of severity depending on how protective the vaccine is. So far, we have only tested the antigen component of our formulations and have seen about 50% increase in protection in hamsters from CDI when immunised. We would expect to see a similar outcome in mice. We expect to see enhanced protection when the antigen is carried on one of our vaccine vehicles (platforms). The aim is to develop the formulation that gives 100% protection in both rodents.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The gut specificity of C. difficile (in particular, the requirements for an anaerobic environment and the availability of bile salts for germination of spores) has meant that suitable non-animal alternatives have so far not been identified.
The information that can be obtained from established tissue culture assays is limited as the infection process involves complex dynamic interactions between the bacterium and host such that C. difficile can undergo each stage of pathogenesis (germination of spores, colonisation of the intestine, secretion of toxins and sporulation) which are impossible to model effectively in non-animal alternatives at present.
Our vaccines require the generation of specific adaptive immune responses (antigen-specific secretory IgA and IgG) in the small intestine. Living animals are required to generate humoral immune responses.

Which non-animal alternatives did you consider for use in this project?

We have experimented with Galleria mellonella (wax moth larvae) in an attempt to replace the use of animals. This invertebrate model is widely used to study different bacterial-host interactions. There is potential for this model to be used to study colonisation by C. difficile following oral administration. I have discussed this idea with colleagues who are focused on developing alternative invertebrate models.

Discussions are on-going with another colleague to use their in vitro gut models and ethical approval is being sought in a separate collaboration to set up an ex-vivo gut model. However, these models will only be useful for investigating the blocking of colonisation of C. difficile by intestinal fluid and sera from vaccinated animals and therefore they do not present a non-animal alternative.

Why were they not suitable?

Oral inoculation with C. difficile results in a degree of infection, however since the intestinal tract of the wax worm differs significantly from that of higher vertebrates, the full spectrum of disease symptoms is not observed in the Galleria model.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Only small group sizes are needed for pilot studies. Based on our previous studies and based on the literature, we estimate all immunogenicity pilot studies (Protocol 1) and all challenge pilot studies (Protocol 2) will require 4 animals for the negative control groups (placebo group and control vaccine bench-mark group) and 4 animals for each experimental group which equates to 24 animals per study. Protocol 1 will be performed twice; the first time to compare different doses (concentrations) of formulation, the second time to compare different ratios of antigens in the formulation. For some vaccines, two routes of immunisation will be compared (oral and nasal). Protocol 2 pilot studies will be performed once, based on the optimised formulation from Protocol 1 studies and the study repeated with group sizes increased if an obvious protective effect of the vaccine is observed.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I had a meeting with the NC3Rs Regional Programme Manager to discuss the protocols outlined in this application. Using, the EDA for Protocol 2, each step was discussed and mapped out to create a flow diagram. We discussed the rationale for the animal numbers based on previous pilot studies we have conducted and the method of randomisation and blinding to limit any bias and achieve greater accuracy.
When possible, two studies will be conducted in parallel using common control groups (placebo and benchmark groups) to reduce the number of animals. The EDA analysis tool will be used to analyse our data.
For planning larger scale challenge studies after these pilot studies, specific group sizes will be determined using online calculation tools such as G*POWER3.1 and professional statistical advice sought from statistician to ensure the minimal but relevant number of animals are used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

By using antigens known from the literature to elicit some protection, assessing them using computer programmes and testing them in the lab for antibody recognition using blood from C. difficile patients, before commencing animal studies we are confident about our choice of antigens in our formulations.
Personnel will be trained to administer doses competently which will reduce variability and therefore reduce the

number of animals needed in each group to achieve more consistent, reliable data.
By performing pilot immunogenicity studies with small group sizes to optimise dose and ratio of antigens, we are confident before we move on to challenge studies (infection with C. difficile) that we have fine-tuned our formulations to give the best possible protection. This means fewer groups of animals need to be vaccinated and infected as we already have some confidence in our formulations. Again a pilot study with small group sizes is performed to initially test the protective efficacy of our optimised formulations. After this, only if we see obvious protection will we go on to test larger group sizes based on power calculations. In this case, we will use the minimal but relevant group sizes to obtain statistically significant data.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

According to a vast body of literature, two well-established clinically relevant animal models will be used for testing the efficacy of our vaccines against CDI. Hamsters and mice are the lowest vertebrates which display the same CDI disease symptoms as those observed in humans. Since disease progression is rapid in these animals, moderate adverse effects are experienced for a minimal length of time (usually 2 to 3 days) before humane end points are reached. Young adults of both rodent species generate similar antibody responses to those observed in humans making them suitable models for testing for immune responses to our vaccines.
For animals being dosed intranasally, general anaesthetic (AB) may be given beforehand to minimise any discomfort and distress. Only a very small volume of formulation (10 microlitres per nostril) will be used for dosing intranasally. The formulation is left to adsorb into the nasal mucosa during anaesthetisation so that the animal does not wake up with fluid in its nose.
For animals given antibiotics by oral gavage, flexible gavage needles will be used and small volumes, typically 50 to 100 microlitres.
For oral gavage of C. difficile spores, typically flexible gavage needles will be used and small volumes, typically 100 microlitres.
By trained, competent personnel carrying out all dosings, minimal discomfort will be experienced and minimal variation incurred.
Animals will be closely monitored after each procedure particularly after infection with C. difficile.
Animals will not experience adverse effects beyond moderate when euthanised at the humane endpoint or experimental endpoint by schedule 1 killing again by trained competent personnel performing this procedure.

Why can’t you use animals that are less sentient?

It is important to use hamsters and mice as these animals are the only known to be clinically relevant for CDI. They must be in their early adult stage of life as, at this stage, they will generate the most robust adaptive humoral immune responses than those younger or much older. It is important that live, well and active animals are vaccinated to be able to generate effective adaptive immune responses, mimicking those achieved in humans.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be handled regularly during their period of acclimatisation (including the refined mouse handling

methods developed for mice) and during the programme of work so that animals are trained to cooperate with procedures avoiding stress. Appropriate housing will include good husbandry practices such as appropriate changing of bedding, housing animals in groups of 2 to 3 per cage until antibiotic treatment prior to challenge studies. Mice are maintained in their groups throughout the study whereas single housing is adopted for hamsters which are solitary to ensure normal behaviour . Enrichment will be provided to allow species-specific behaviours, meeting or exceeding the standards prescribed under ASPA. For example, all animals will be provided with paper nesting material, cardboard tubes and wooden chew blocks in their cages. Hamsters may also be provided with wheels as a way of providing stimulation. Animals will be given free access to food and drinking water at all times.
Frequent welfare checks will be conducted and increased post procedures. Any signs of pain, suffering and distress will be monitored and advice sought from the NVS whenever necessary. To this end the use of established scoring systems will be used to monitor the welfare of the animals throughout the study and schedule 1 killing performed if animals reach the score associated with the humane end point. One way we are considering refining our accuracy in monitoring disease progression and to euthanise at earlier humane endpoints is to use temperature transponders. This practice has been established for the hamster CDI model at other institutes and a drop in core body temperature to 2C considered a suitable early humane endpoint along with weight loss. However the transponders have to be surgically implanted into the abdomen since when embedded subcutaneously into the nape, the readout fluctuates and does not accurately convey core body temperature. Since the adverse effects

associated with surgical implantation may outweigh the early symptoms of CDI, we are not going down this route. However, we are committed to reviewing the use of transponders if more refined implantation methods or devices become available. General anaesthetic will be given for a minimal length of time for animals to be intranasally-vaccinated to prevent distress, as agreed with the NVS, and animals will be allowed to recover fully before being returned to the home cage. Animals will be terminally anaesthetised prior to cardiac blood sampling.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will follow the LASA guidelines for administration of substances. I will regularly check the nc3rs website (www.nc3rs.org.uk) for current guidelines, practical information and to get links to publications and online resources including videos. I will attend seminars given on refinement and discuss refinement with other scientists at conferences to learn best practices.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Maintain regular communication with scientists at other institutes who are conducting similar animal studies and glean helpful information based on their experience. Keep up to date with the literature on C. difficile infection studies, attend conferences, attend general seminars on the 3Rsand stay abreast of literature on the 3Rs with relevance to C. difficile. Refine protocols and procedures accordingly after consultation with Named animal staff, the NC3Rs Regional Programme Manager and with Home Office Inspector where a change to the license is required.

Home Office

NON-TECHNICAL SUMMARY

182. The assessment of analgesic mechanisms to support the development of novel analgesic therapies

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Analgesia, Neuroimmunology, Acute pain, Inflammatory pain, Neuropathic pain

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The aim of this project is to investigate the pathways involved in the sensation of pain and to identify and develop new and effective analgesics (pain killers).
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Chronic pain is the one of the biggest healthcare problems of the 21st century, affecting between 2050% of the adult population worldwide. In 2016, a study investigating the prevalence of chronic pain estimated that between one third to one half of the UK population are affected by chronic pain (Fayaz et al, 2016). Furthermore, chronic pain is also associated with the emergence of other serious healthcare issues, including depression, anxiety, fatigue, sleep disturbances, as well as changes in mental function, which often make the pain worse, severely affecting the physical and mental state of an affected individual, and increasing the demand on healthcare and support services. Across Europe, the economic cost associated with chronic pain has been estimated at around 450 billion euros per year (Breivik et al, 2013). These statistics highlight the need to develop new and effective analgesics for the treatment of chronic pain.
Our current understanding of the mechanisms involved in the development and maintenance of chronic pain are significantly limited, meaning that existing therapies for its treatment are largely unsuccessful, or have significant side effects associated with long-term use. One of the most prescribed classes of analgesics used in the treatment of chronic pain are opioids, however, in recent years they have been associated with significant levels of misuse and were responsible for 42,249 deaths in the US alone in 2016 (National Institute on Drug Abuse, US), highlighting the need for new non-opioid therapeutic options. In addition to chronic pain, there is also a substantial unmet need in the treatment of acute pain, specifically post-operative pain, which is commonly treated with opioid-based therapies.
Importantly, pre-operative opioid use is an important risk factor for persistent/chronic post-operative use and is linked to longer opioid prescriptions. Therefore, in addition to chronic pain conditions, there is also significant societal value in developing effective, non-opioid analgesics for the treatment of acute pain conditions.
This project aims to study the mechanisms involved in the development of acute and chronic pain conditions, in order to facilitate the development of new and effective analgesic therapies.
References:
Breivik et al. 2013. BMC Public Health.13:1229
Fayaz et al. 2016. BMJ Open. doi.org.10.1136/bmjopen-2015-010364.
What outputs do you think you will see at the end of this project?

The outputs from this project can be summarised by the following:

- New information: Outputs from this project will enhance our understanding of the mechanisms involved in pain, with the intent of facilitating the development of new and effective analgesics that have an improved benefit-risk profile over existing therapies.

- Publications: Our aim is to publish outputs from this project in open-access peer reviewed scientific journals.

- Products: The principal aim of this project is to further understand the mechanisms involved in the development and maintenance of pain in order to develop and produce new and effective analgesic therapies.

Who or what will benefit from these outputs, and how?

The overall aim of this programme of research is to directly benefit individuals currently suffering from pain through the identification and development of new analgesic therapies.
The short- and medium-term benefits of this work are to support the ongoing therapy development programmes at the establishment in order to test the analgesic potential of new immune cell-related therapies for the treatment of pain conditions (0-5 years).
The long-term benefits of this work include the identification of novel immune cell-related targets that are involved in the development and maintenance of pain conditions, which will form the basis of a continued effort to develop novel and effective analgesics (5-10 years).

How will you look to maximise the outputs of this work?

Where possible, every effort will be made to publish and share relevant outputs of these studies (e.g. identified mechanisms involved in the development/maintenance of pain) with the wider scientific community. This will be through scientific collaboration with external academics, as well as the publication of research findings in open access research journals. Where possible, interim updates will be presented at local and/or international conferences.

Species and numbers of animals expected to be used

- Mice: 6300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project aims to identify and develop novel analgesics (pain killers) for the treatment of chronic pain conditions. In order to assess the ability for a new drug to reduce pain, we first need to create a situation where there is pain to treat. As pain is a conscious sensation, this assessment needs to take place using a conscious animal. Within this project licence, we propose to use adult mice to assess the effectiveness of new analgesics. Mice display very similar behaviours (i.e. hypersensitivity to environmental stimuli) to humans with respect to pain, and their genetics can be readily altered in order to understand the mechanisms by which an analgesic may be working. Furthermore, there is a high level of translatability of the mechanisms that underlie painful conditions between mice and humans.
For example, the functional loss of a specific gene that causes pain insensitivity in humans (e.g. Scn9a), can also lead to pain insensitivity in mice. In addition, widely used analgesics used to treat human pain, such as aspirin, ibuprofen, gabapentin and opioids, are also highly effective at alleviating pain in mice.

Typically, what will be done to an animal used in your project?

An animal used under this project licence will typically experience the following:

- **The basal assessment of motor function and sensory sensitivity (protocol 1).** Within this protocol, the locomotor activity (ability to move) of an animal will be assessed, along with its ability to respond to a heat, cold and/or touch stimulation. All methods of assessment used within this project allow an animal to withdraw from testing at any time, and suitable maximum exposure periods are used to ensure that no

animal will be harmed by any of the assessments. An animal will be limited to two types of assessment per day (Max. 10 assessments per modality, over a maximum of 35 days), and will only undergo this protocol once. As part of this assessment, an animal is likely to receive a single course of treatment (administered by an injection, through topical application or provided within the food/water).

OR

- **The induction and assessment of inflammatory pain (protocol 2).** The induction of inflammatory pain will involve the injection of a chemical substance that causes inflammation. This will only occur once. Inflammation will last up to 14 days (depending on the chemical substance injected), and within this time an animal is likely to receive a single course of treatment (administered by an injection, through topical application or provided within the food/water). The presence of inflammation is expected to make an animal more sensitive to heat, cold and touch stimulation, which can be assessed for up to 14 days (depending upon the chemical substance injected). The effect of any given treatment to reduce pain will be assessed by measuring the time taken for an animal to withdraw from a specific sensory stimulus (e.g. heat, cold or touch). An animal will be limited to two types of assessment per day and will only undergo this protocol once.

OR

- **The induction and assessment of neuropathic pain (protocol 3).** The induction of neuropathic pain will involve the animal to undergo a brief surgical procedure, under general anaesthesia, in order to tie/cut part of the sensory nerve innervating the hind leg of the animal. Following this procedure, an animal becomes more sensitive to cold and touch stimulation, which can be assessed for up to 35 days. As part of this assessment, an animal is likely to receive a single course of treatment (administered by an injection, through topical application or provided within the food/water). The effect of any given treatment to reduce pain will be assessed by measuring the time taken for an animal to withdraw from a specific sensory stimulus (e.g. cold or touch). An animal will be limited to two types of assessment per day and will only undergo this protocol once.

OR

- **The induction and assessment of acute colorectal distension (protocol 4).** In this protocol, an animal will be withheld food overnight prior to the model being implemented. Under terminal anaesthesia, a balloon catheter will be inserted into rectum of an animal, and will undergo a series of short inflation cycles (inflation and deflation), which in turn will cause the colorectal region of the mouse to distend and recover. Once these cycles are complete, the animal will be immediately euthanised and tissues will be collected. As part of this assessment, and prior to model implementation, an animal is likely to receive a single course of treatment (administered by an injection, through topical application or provided within the food/water). The effect of any given treatment to potentially reduce pain will be assessed by measuring specific markers of cellular activity in tissues of the nervous system.

What are the expected impacts and/or adverse effects for the animals during your project?

The main adverse effect associated with this project is pain caused by inflammation or nerve injury.
The local injection of an inflammatory agent is likely to cause short lasting pain and hypersensitivity to specific sensory stimuli, as well as local swelling (oedema) and redness of the treated area. Where an injection is given at the paw or knee, animals may also exhibit reduced use of the affected limb immediately after injection, however, this should subside within hours. The majority of inflammatory agents to be administered are likely to only cause a mild inflammatory reaction, which should minimise the level of discomfort and pain experienced by the animal.
The tying or cutting of sensory nerves innervating the rear leg is likely to cause mild to moderate spontaneous pain, in addition to an increased sensitivity to specific sensory stimuli. Immediately after the induction of any nerve injury model, local inflammation is expected at the site of nerve injury as well as the site of surgery. An animal that has undergone this procedure is likely to show a reduction in affected limb use, although this should not affect normal rearing behaviour or feeding. These adverse effects are expected to last for the duration of the

model (up to 35 days). Any animal that has undergone sham surgery is likely to display mild and short-lasting signs of hyperalgesia associated with the local inflammation caused by sciatic nerve exposure. These adverse effects are not expected to last more than seven days.
Colorectal distension will be performed under terminal anaesthesia, therefore pain, suffering and distress is not expected.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Of the animals used under this licence, it is estimated that approximately 60% will experience mild severity, and 40% of animals will experience moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project aims to identify and develop novel analgesics for the treatment of chronic pain conditions. In order to assess the ability of new therapies to reduce pain, we first need to create a situation where pain is measurable, and therefore treatable. Importantly, pain is a conscious sensation, involving the integration of multiple molecular mechanisms across peripheral and central nervous systems. For this reason, the assessment of pain needs to be performed on sentient, behaving animals, with developed peripheral and central nervous systems.

Which non-animal alternatives did you consider for use in this project?

Suitable *in vitro* models have been considered to investigate whether a novel therapy/therapeutic target can directly affect isolated cellular processes involved in the processing of noxious stimuli. Examples of such models include the assessment of relevant cultured cell types (e.g. sensory neurons, astrocytes, immune cells) using a variety of methods (e.g. electrophysiology, calcium imaging, peptide release assays, transcriptomic analysis). Although these models do not model pain, they may indicate whether a therapy/therapeutic target can directly, or indirectly, affect mechanisms involved in the detection of noxious stimuli. Results from these studies will help in predicting the analgesic potential, in addition to the potential mechanism of action, of a novel therapy/therapeutic target before being tested *in vivo*.

Why were they not suitable?

While these models do offer some value in predicting the potential analgesic effect of a novel therapy, they do not model pain. Pain is a conscious behaviour, involving the integration of numerous genetic, molecular and cellular factors, within, and across, functional peripheral and central nervous systems. Given this level of complexity, to date, the mechanisms involved in the development and/or maintenance of chronic pain are poorly understood, and as such, there are no suitable alternatives to faithfully model pain behaviour *in vitro*.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals to be used under this licence has been estimated through the anticipated need, over a five-year period, to assess, develop and identify novel therapies/therapeutic targets for the treatment of different pain conditions within the licensed establishment. This estimation has been based on previous experience gained working under other pain-focused project licences at other licensed establishments within the United Kingdom.
Statistical advice has also been sought to determine the number of animals needed for each experimental model to ensure sufficient power for the measured endpoints and hypotheses being tested.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Statistical advice is always sought as part of the study design process to ensure that the minimum number of animals is used to sufficiently meet to requirements of the experimental aim. Where appropriate, historic data will be used to support the experimental design process, however, if suitable data are not available, small pilot studies will be undertaken, under statistical guidance.
In addition to statistical support, all studies conducted under this licence will undergo internal peer review in order to ensure that all aspects of experimental design are suitable for the study being proposed.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Under this licence, the following measures will be taken to optimise animal use:

- *In vitro* cell culture systems will be used to analyse specific aspects of sensory physiology in isolation.
- Pilot studies will be used to ensure the effective design of experiments, and to keep the number of animals used to a minimum.
- Where appropriate, at the end of an experiment, spare tissues will be freely shared with other researchers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Methods of pain assessment: All methods of pain assessment described within this protocol have been designed to assess the threshold at which an animal withdraws from a given stimulus. The use of withdrawal thresholds to assess pain behaviour, means that an animal is free to withdrawal from any given stimulus at any

time, keeping the amount of pain, distress and lasting harm experienced by the animal to the absolute minimum, while still having utility in measuring pain behaviour. In addition, these methods are the least invasive to assess sensitivities to different sensory modalities, including mechanical, heat and cold. The use of these different sensory modalities enables the investigation into different types of pain that involve different signalling pathways, meaning that we can assess multiple pain pathways while using as few animals as possible. Importantly, suitable cut-offs/limits are put in place on all experimental models/methods in order to prevent damage and minimise distress to the animal.
Models of inflammatory pain (injection of pro-inflammatory substances): All of the inflammatory models proposed are well validated and widely published, with reproducible and reliable associated endpoints. The proposed models also cover various aspects of inflammation, which differ in their mechanism of action, duration of action, and effect on the animal. Importantly, these models allow for the assessment of specific, clinically relevant inflammatory pathways to be investigated. Furthermore, clinically relevant anti-inflammatory analgesics (e.g. NSAIDs) are effective in attenuating the pain behaviours induced by these models, highlighting the translatability of these models in assessing analgesic effectiveness of novel therapies/therapeutic targets for the treatment of inflammatory pain.
Models of neuropathic pain (sciatic nerve injury): All of the neuropathic pain models proposed are well validated and widely published models, with reproducible and reliable associated endpoints. Importantly, the models proposed elicit clinically relevant phenotypes commonly associated with neuropathic pain, such as sensory hyperalgesia and allodynia. In addition, the pain behaviours associated with the proposed models are resistant to commonly used anti-inflammatory analgesics (e.g. NSAIDs), which is a common hallmark of neuropathic pain in patients. Furthermore, therapies that are effective at treating neuropathic pain in the clinic (e.g. gabapentin, lidocaine, opioids) are also effective at alleviating the pain symptoms associated within the proposed models, highlighting their translatability in modelling clinically relevant neuropathic pain.
Models of acute visceral pain (colorectal distension): The model of acute visceral pain proposed is well validated and widely published, with reproducible and reliable associated endpoints. This model will be performed under terminal anaesthesia and assesses the activity of damage-sensing sensory neurons (nociceptors) that innervate the intestine. The activity of these neurons is correlated to the pain experienced by patients with visceral pain disorders (e.g. inflammatory bowel disease), and therefore understanding how therapies can affect these neurons will help with the development of therapies for treating visceral pain.

Why can’t you use animals that are less sentient?

Pain is a complex, conscious and integrative behaviour, relying upon the activation and integration of numerous neuronal pathways and cellular interactions. As such, pain behaviour needs to be modelled in awake animals with fully developed peripheral and central nervous systems.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The models described within this licence will undergo continual review and refinement to ensure that they cause the minimum harm to animals, while maintaining scientific utility. For example, the duration of model implementation will be continually reviewed to ensure that the earliest appropriate endpoint is used. Furthermore, the number and frequency of behavioural assessments will be kept to the minimum number needed for the study.
For each inflammatory and neuropathic model, animals will be assessed daily and monitoring score sheets will be followed to assess the welfare of the animal as the model progresses.
Current good practice in animal handling (e.g. using tunnels to move mice) will be reviewed and implemented where practicable. In addition, consideration will be taken with respect to the enrichment included within the cage along with specialised bedding (e.g. softer bedding), to minimise the welfare costs. Warming cabinets will be used for post-surgery recovery, and wet food/mash will be provided to ensure all animals recover as quickly as possible. Additional refinements to the cage environment and animal handling will be considered throughout the duration of this licence.

The use of home cage monitoring (e.g. Tecniplast DVC or Vium-based platforms) will be investigated in order to improve pain behaviour assessment. These platforms allow for a non-invasive assessment of animal behaviour within the home cage environment, and therefore have the potential to minimise welfare costs further, as long as the observed behaviours recorded are robust, reproducible and translatable with respect to monitoring pain.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The following is a list of the best practice guidance that we routinely follow.

- Deuis JR et al., (2017) Methods used to Evaluate Pain Behaviours in Rodents. Frontiers in Molecular Neuroscience 10:284. Diehl KH et al., (2001) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes: JOURNAL OF APPLIED TOXICOLOGY 21, 15–23
- Hestehave et al. (2017) Is there a reasonable excuse for not providing post-operative analgesia when using animal models of peripheral neuropathic pain for research purposes. Plos One 12(11):e0188113.
- LASA - Guiding principles on good practice for Animal Welfare and Ethical Review Bodies Sep 2015
- LASA - Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2017
  Mogil J. (2019). The translatability of pain across species. Phil Trans Roy Soc.
- Morton DB et al., (1993) Removal of blood from laboratory mammals and birds Laboratory Animals 27, 1-22
- NC3R's - Responsibility in the use of animals in bioscience research: Expectations of the major research council and charitable funding bodies
- Prescott MJ, Lidster K (2017) Improving the quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156.
- Review of harm-benefit analysis in the use of animals in research - Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group chaired by Professor Gail Davies Nov 2017

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in the 3Rs through regular referral to the NC3R's website, as well as other published literature. Furthermore, the establishment Named Information Officer (NIO) will facilitate the dissemination of information in relation to any such advances. In accordance with any updates, we will review and revise the protocols within this licence to ensure they have been adequately considered, and where applicable, applied.

Home Office

NON-TECHNICAL SUMMARY

183. The cellular and synaptic basis of depression and anxiety

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

depression, anxiety, early life adversity, fragmented maternal care, hippocampus

Animal types	Life stages
Mice	juvenile, adult, neonate, embryo, pregnant
Rats	juvenile, adult, neonate, embryo, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to increase our understanding of the ways in which changes in the activity of neurons and the synapses by which they communicate can lead to depression and anxiety. The mechanism of action of existing antidepressant medications is poorly understood in many cases, and a parallel aim is to improve this understanding, and to identify novel therapeutic strategies. A particular focus will be the impact of early life adversity, and how this impact can be mitigated.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

There is a growing awareness of the key role of early life adversity as a risk factor for the development of psychiatric disorders in adulthood. Experience-dependent changes in the brain, as well as being involved in memory formation, also contribute to the development of mood disorders and anxiety. By understanding these changes, we may be able to identify novel therapeutic strategies for the treatment of these conditions.

What outputs do you think you will see at the end of this project?

Depressive disorders are the biggest single contributor to chronic disability worldwide. Despite many years of research, there has been limited progress in the development of new treatments in recent decades. Currently available drugs have limited and variable efficacy, and, when effective, typically take weeks or months to improve mood and behaviour. The problem stems, in part, from our lack of understanding of the brain mechanisms underlying depression, and uncertainty surrounding the ways in which antidepressants work, topics that we plan to address in this project. Regarding anxiety, currently available treatments often cause sedation and cognitive impairment as well as reducing anxiety. As part of a wider collaborative research effort, we are currently working on the development of non-sedating anti-anxiety drugs. The main outputs of this project will be new information about the brain mechanisms of depression and anxiety, and information about new drugs that might be useful to treat these conditions. Our work will mainly be communicated in the form of research publications, but also in seminars and conference presentations.

Who or what will benefit from these outputs, and how?

Our collaborators, and researchers working in related fields, will benefit from our research both during and beyond the time-frame of the project, as a result of informal information sharing, conference presentations, and reading our peer-reviewed research publications. This information will help to shape the research programmes of other researchers by alerting them to potential new avenues of research, preventing unnecessary duplication of work, and informing them of unsuccessful approaches. Longer-term benefits may include the development of potential new drugs for human clinical trials, or the identification of new strategies for future drug development. But these benefits—such as the development of a new drug or therapy—will occur over a timescale of several years and are likely to be realised beyond the lifetime of the licence itself.

How will you look to maximise the outputs of this work?

The work detailed in this project involves collaborations with other research groups. By bringing a variety of

sources of expertise and experimental approaches to bear on the project, we aim to maximise the visibility of the research publications and new knowledge that will be generated. If an experiment produces data that answer the question posed, they will be published, regardless of whether the result is positive or negative. Details of unsuccessful approaches will also be shared informally in discussions with colleagues and other researchers in the field, and at scientific conferences.

Species and numbers of animals expected to be used

- Mice: 2400
- Rats: 1600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The animals used in this project will be rats and mice. The brain regions that are thought to be involved in anxiety and depression in humans have clear structural and functional parallels to the same brain regions in rats and mice, something that is not true of non-mammalian or invertebrate species. Like humans, rodents that experience early life adversity show behavioural changes in adulthood, including depressive-like symptoms in behavioural tasks. In order to study the impact of early life adversity, neonatal animals will experience an interruption of maternal care. This will be caused by providing a reduced amount of nesting material, leading the mother to make more frequent trips away from the nest. The behavioural and physiological impact of this experience will be assessed in juvenile and adult animals.

Typically, what will be done to an animal used in your project?

In many cases, animals will be provided with a reduced amount of bedding during early postnatal development. This will cause the mother to make more frequent trips out of the nest, resulted in fragmented maternal care, a form of early life adversity. The effects of this experience on behaviour and brain function will then be assessed when the animals reach adulthood. Animals will often be injected with non-harmful drugs in conjunction with behavioural testing (typical duration = 1-4 weeks). In some cases, animals will be surgically implanted with electrodes for the direct recording of brain activity, either during behavioural testing, or under anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

Fragmented maternal care will result in a slight reduction in body weight throughout development, and long-lasting, but subtle, behavioural changes. The procedure will not cause pain or lasting distress. Administration of drugs may cause mild and temporary changes in behaviour (< 24 hours). Behavioural tests will cause no lasting adverse effects or distress. Surgical procedures, such as the implantation of electrodes into the brain, may cause pain, but this will be controlled using surgical anaesthesia, and postoperative analgesia as required. Animals may also experience some postoperative weight loss, but these signs should last no more than 3 days.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximate proportions in each severity category will be as follows:

Mice:
mild: 55% moderate:

35% non-recovery:

10%

Rats:
mild: 30% moderate:

30% non-recovery:

40%

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The main aim of this project is to study the physiological basis of anxiety and depression-like symptoms, and the actions of antidepressant drugs that may improve these symptoms. This is not possible without the use of animals in which behavioural changes--or changes in brain circuits relevant to these behaviours--can be studied. This approach is not possible in human participants owing to the invasive nature of the techniques required to measure brain activity and plasticity.

Which non-animal alternatives did you consider for use in this project?

Human electrophysiological techniques such as EEG recording (recording oscillatory brain activity from the scalp), and brain imaging such as functional magnetic resonance imaging (fMRI), have provided valuable information about the brain areas involved in anxiety and depression.

Why were they not suitable?

Although the use of EEG and fMRI in human participants can reveal correlations between brain activity and behaviour, mood, and cognition (e.g. problem-solving and memory), there are some critical drawbacks. The central problem is the low spatial resolution of these techniques--meaning that the techniques cannot identify the molecular and cellular mechanisms responsible for behaviour, only the general areas of the brain that are active. It is impossible to study processes that occur at the level of the cell, including the ways that neurons communicate with other cells around them, without the without the use of invasive techniques that involve surgery and cannot be employed in healthy humans.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers are based on an estimate of the numbers required to achieve statistically robust effects across all of the work laid out in the licence. In some cases, this includes the numbers of animals needed for breeding programmes to obtain the experimental animals. The estimate of numbers is based on past experience of similar experiments, the published literature, and, in some cases, on preliminary data.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where possible, we have designed experiments to minimise the impact of variables responsible for trial-to-trial or between-animal variability. For example, during behavioural tasks, animals will be handled by trained staff. They will also be allowed to get used to being handled, the person handling them, and the room in which behavioural testing will be carried out. Handling techniques that cause minimal stress will be used, such as cupping and tube-handling. Behavioural testing is conducted in quiet rooms away from human activity. Reducing variability by doing these things means that fewer animals are needed to achieve statistically significant results. In many cases, we will collect multiple measures in the same animal, either by repeating behavioural tests to increase reliability, or by recording multiple electrophysiological measures of the impact of a drug. It will often be possible to compare a measure before and after the administration of a drug, reducing the need for comparisons between different animals, and hence reducing the numbers required.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will also minimise the numbers of animals used by conducting pilot studies when the effects of a treatment or manipulation are unknown. A pilot study will allow us to estimate the numbers needed to observe statistically robust effects in the main experiment, avoiding the problem of underpowered studies if the numbers are too low (and there is therefore not enough data to tell whether the change we are interested in has occurred), or unnecessary use of animals if the numbers are two high. Where animals are bred in-house, we will use efficient breeding programmes that generate the required numbers of animals and no more.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The animals used in this project will be wild-type rodents (i.e. normal animals with no genetic alterations) or animals with non-harmful genetic alterations (changes in genes that do not result in obvious clinical signs, but only subtle behavioural changes that can be detected using specific tests). Some of these animals will undergo a form of fragmented maternal care known as the "reduced bedding model", in which a reduced amount of nesting material is provided for a number of days starting from the second day after pups are born. Reducing the amount of bedding causes the mother to enter and leave the nest more frequently than usual, resulting in an interruption of maternal care. This causes a range of phenotypic (behavioural and cellular) changes in adulthood that are consistent with the impact of early life adversity in humans. These include increased anxiety, increased depressive symptoms, and an increased risk of the development of drug addiction. For these reasons, we believe that we can use this procedure to increase our understanding of the changes in brain activity and plasticity (changes in the wiring of the brain resulting from experience) associated with this model, by studying the impact of antidepressant drugs in animals that have undergone the procedure.
Fragmented maternal care, has a mild to moderate rating and is considered less stressful than an alternative approach involving complete physical separation of the dam and pups. Previous experience demonstrates that a consequence of fragmented care can be a reduction in the speed that pups grow. However, the pups' feeding is normal, and they appear healthy, despite being slightly smaller at weaning.
Some of the work in this project will be carried out in brain tissue taken from animals that have been painlessly euthanised, or in animals under terminal anaesthesia (anaesthesia from which an animal does not wake up) that also suffer no pain or distress. However, while these techniques can provide information about the functioning of neurons, synapses, and brain circuits, they cannot alone establish the relationship between brain activity and behaviour. For this, it is necessary to record from animals in which electrodes have been implanted into specific areas of the brain under anaesthesia, before the animal is allowed to wake up so that, after recovery from surgery, we can investigate how the activity of neurons changes with different behaviours.
The animals will receive painkillers after surgery, as advised by a vet, they will be closely monitored, and they will be given other supportive measures such as being kept warm and provided with special food. Recording of brain activity will not begin until the animals have fully recovered from surgery.

Why can’t you use animals that are less sentient?

The brain regions involved in anxiety and even depression-like symptoms in rats and mice work in a similar way to the same regions in humans, something that is not true of non-mammalian or invertebrate systems. We can find out a lot of information by looking at the functioning of neurons and synapses in brain tissue taken from animals that have been painlessly euthanised, or in animals that are asleep under an anaesthetic from which they do not wake up (terminally anaesthetised). A substantial part of the work outlined in this project will be carried out in this way. However, while these techniques can tell us a lot about the functioning of neuronal circuits, they cannot alone establish connections between brain activity and behaviour.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All staff involved in animal experiments will be trained in handling techniques that minimise stress, and will receive extensive training in all other techniques with which they will be involved. All animals undergoing surgical procedures will receive postoperative pain management and will be closely monitored during recovery. During the project, we will continually seek to identify refinements to our animal husbandry and experimental techniques, and implement these as appropriate. For example, advances in electrophysiological techniques, such as the ability to record simultaneously from multiple sites in the brain, are making it possible to collect more information from each animal. We will adopt techniques such as this where feasible.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow best practice as laid out in guidance documents published by the UK Home Office and NC3Rs. Our reporting of research findings will follow the NC3Rs ARRIVE 2.0 guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep informed about advances in the 3Rs via the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs). The Named Veterinary Surgeon and staff of REDACTED will also inform us of improvements in experimental and animal husbandry techniques, and we will implement these as appropriate. We will also identify relevant refinements to our techniques in the published scientific literature, and will seek to implement these where feasible.

Home Office

NON-TECHNICAL SUMMARY

184. The cellular and synaptic basis of learning and addiction

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

learning, memory, synaptic plasticity, neurodevelopmental disorders, addiction

Animal types	Life stages
Mice	adult, juvenile, pregnant, embryo, neonate
Rats	juvenile, adult, embryo, neonate, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs

it's addressing.

What's the aim of this project?

The aim of this project is to increase our understanding of the mechanisms that underlie the formation of memory—changes in the activity of neurons and the synaptic connections by which they communicate. The project focusses on the normal mechanisms of learning, their disruption by neurodevelopmental abnormalities, and the formation of maladaptive memories in addiction.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This work will provide new information about the mechanisms of normal memory formation, and the ways in which these mechanisms are affected by neurodevelopmental disorders such as fragile-X syndrome. Experience-dependent changes in the brain also contribute to the development of drug addiction. By understanding these changes, we may be able to identify novel therapeutic strategies for the treatment of memory impairment and addiction.

What outputs do you think you will see at the end of this project?

The main outputs of this project will be new information regarding the neural and synaptic basis of learning and memory, as well as some of the maladaptive changes that occur in animal models of human disorders such as fragile X syndrome and addiction. These outputs will primarily be disseminated in the form of research publications, but also in seminars and conference presentations.

Who or what will benefit from these outputs, and how?

Our collaborators, and researchers working in related fields, will benefit from these outputs both during and beyond the time-frame of the project, as a result of informal information sharing, conference presentations, and reading our peer-reviewed research publications. This information will help to shape the research programmes of other researchers by alerting them to potential new avenues of research, preventing unnecessary duplication of work, and informing them of potentially unsuccessful approaches. Longer-term benefits will include the in vivo validation of potential therapeutic strategies, and the identification of possible new strategies for drug development. All the objectives of this project have a translational or drug-discovery component, and we anticipate that our work will inform future preclinical and clinical research programmes. But these benefits—such as the development of a new drug or therapy—will occur over a timescale of several years and are likely to be realised beyond the lifetime of the licence itself.

How will you look to maximise the outputs of this work?

The work detailed in this project involves collaborations with other researchers, both locally and in other labs around the world. By bringing a variety of sources of expertise and experimental approaches to bear on the project, we aim to maximise the visibility of the research outputs that will be generated. If an experiment produces data that answer the question posed, they will be published, regardless of whether the result is positive or negative. Details of unsuccessful approaches will also be shared informally in discussions with colleagues and other researchers in the field, and at scientific conferences.

Species and numbers of animals expected to be used

- Mice: 4370
- Rats: 1570

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The animals used in this project will be rats and mice. The brain regions involved in learning and memory in both species have clear structural and functional parallels to the same brain regions in humans, something that is not true of non-mammalian or invertebrate systems. In many cases, rats will be used owing to their proficiency and flexibility in a wide range of behavioural tests. In other cases we will use mice; these are capable of most of the same behavioural tasks that can be carried out using rats, and they offer the possibility of a greater range of genetic manipulations that is currently available in rats. In most cases, we will focus on juvenile or young adult animals, old enough to show robust performance in memory tasks.

Typically, what will be done to an animal used in your project?

Animals will typically be injected with non-harmful drugs in conjunction with testing in one or more behavioural tasks. This will usually continue for 1-4 weeks. In some cases, animals will be surgically implanted with electrodes for the direct recording of brain activity, either during behavioural testing, or under anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

Administration of drugs may cause mild and temporary changes in behaviour (< 24 hours). Most behavioural tests will cause no adverse effects or distress, but the water maze task that involves swimming to find an escape platform may cause temporary stress. Surgical procedures, such as the implantation of electrodes into the brain, may cause pain, but this will be controlled using surgical anaesthesia, and postoperative analgesia as required. Animals may also experience some postoperative weight loss, but these signs should last no more than 3 days.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately 70% of animals will experience a mild severity category, 20% moderate, and the remaining 10% will undergo non-recovery procedures only. The proportions will be similar for rats and mice.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

A central purpose of this project is to study the physiological basis of memory and addiction, and this is not possible without the use of animals that can perform behavioural tasks. A major focus is the recording of neuronal and synaptic activity, and this is not possible in healthy human participants (see below).

Which non-animal alternatives did you consider for use in this project?

Human electrophysiological techniques such as EEG recording, and functional imaging such as fMRI, can provide information about which brain areas are active when someone carries out a specific behaviour. This avoids the use of animals, and these techniques have been very informative.

Why were they not suitable?

Although the use of EEG and fMRI can reveal correlations between brain activity and cognition, there are some drawbacks: first, the spatial resolution of these techniques is poor, and it is impossible to study cellular and synaptic processes without the use of invasive techniques that cannot be employed in humans. Second, EEG and functional imaging alone cannot establish whether activity in a specific brain region plays a causal role in the behaviour observed, or indeed is necessary for that behaviour.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers are based on an estimate of the numbers required to achieve statistically robust effects across all of the work laid out in the licence. In some cases, this includes the numbers of animals needed for breeding programmes to obtain the experimental animals. The estimate of numbers is based on past experience of similar experiments, the published literature, and, in some cases, on preliminary data.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where possible, we have designed experiments to minimise the impact of variables responsible for trial-to-trial or between-animal variability. For example, during behavioural tasks, animals will be handled by trained staff, and habituated to handling and the testing environment prior to the assessment of behaviour. Handling techniques that cause minimal stress will be used, such as cupping and tube-handling. Where possible, behavioural testing will be conducted in rooms free from excessive noise or disturbance. Reducing variability means that fewer animals are needed to achieve statistically significant results.

In many cases, we will collect multiple measures in the same animal, either by repeating behavioural tests to increase reliability, or by recording multiple electrophysiological measures of the impact of a drug. It will often be possible to compare a measure before and after the administration of a drug, reducing the need for between-subjects comparisons, and hence reducing the numbers required.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will also minimise the numbers of animals used by conducting pilot studies when the effects of a treatment or manipulation are unknown. A pilot study will allow us to estimate the numbers needed to observe statistically robust effects in the main experiment, avoiding the problem of underpowered studies if the numbers are too low, or unnecessary use of animals if the numbers are two high. Where animals are bred in-house, we will use efficient breeding programmes that generate the required numbers of animals and no more.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Because rats and mice have homologous brain structures to those in humans, it is possible to study behavioural processes such as learning and memory in these animals, without the need to use 'higher' mammalian species. Most of the animals tested in this project will be wild-types or animals with nonharmful genetic alterations. An exception is the FMR1 knockout mouse, an animal model of the neurodevelopmental abnormalities evident in Autism Spectrum Disorder. These animals have an increased risk of developing sound-induced epileptic seizures as they grow older. For this reason, these animals will be kept in a quiet environment, and testing will be carried out at the earliest age possible.

A large amount of the work in this project will be carried out in brain tissue taken from animals that have been painlessly euthanised, or in animals under terminal anaesthesia that also suffer no pain or distress. However, while these techniques can provide information about the functioning of neurons, synapses, and brain circuits, they cannot alone establish the relationship between brain activity and behaviour. For this, it is necessary to record from animals in which electrodes have been chronically implanted. This will be carried out under general surgical anaesthesia, and the animals will receive postoperative pain management, close monitoring, and other supportive measures as required. Recording of brain activity will not begin until the animals have fully recovered from surgery.

Why can’t you use animals that are less sentient?

The brain regions involved in learning and memory in rats and mice have clear functional parallels to the same regions in humans, something that is not true of non-mammalian or invertebrate systems. In most cases, our research requires the use of young adult animals in which memory-related brain circuitry and behavioural abilities are fully developed. However, a component of our work will involve the testing of the neurodevelopmental abnormalities in a mouse model of autism spectrum disorder. In this case, a substantial part of the work will be carried out using tissue from animals in the first weeks of life. It is also possible to study the functioning of neurons and synapses in brain tissue taken from animals that have been painlessly euthanised, or in animals that have been terminally anaesthetised. A substantial part of the work outlined in this project will be carried out in this way. However, while these techniques can tell us a lot about the functioning of neuronal circuits, they cannot alone establish connections between brain activity and behaviour.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All staff involved in animal experiments will be trained in handling techniques that minimise stress and will receive extensive training in all other techniques with which they will be involved. All animals undergoing surgical procedures will receive postoperative pain management and will be closely monitored during recovery. During the project, we will continually seek to identify refinements to our animal husbandry and experimental techniques and implement these as appropriate. For example, advances in electrophysiological techniques, such as multi-site recording, are making it possible to collect more information from each animal. We will adopt techniques such as this where feasible.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow best practice as laid out in guidance documents published by the UK Home Office and NC3Rs. Our reporting of research findings will follow the NC3Rs ARRIVE 2.0 guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep informed about advances in the 3Rs via the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs). The Named Veterinary Surgeon and staff will also inform of improvements in experimental and animal husbandry techniques, and we will implement these as appropriate. We will also identify relevant refinements to our techniques in the published scientific literature and will seek to implement these where feasible.

Home Office

NON-TECHNICAL SUMMARY

185. The Development of Animal Health Products

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
  - (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Animal Health, Veterinary, Feed additives, Farm animals, Companion animals

Animal types	Life stages
Rats	adult
Mice	adult
Beagles	adult, juvenile
Cattle	juvenile, adult, pregnant

Animal types **Life stages**

Sheep	juvenile, adult, pregnant
Pigs	juvenile, adult, pregnant, neonate
Broiler Chickens, Laying Hens, Turkeys	neonate, juvenile, adult, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The intention of the work covered by this licence is to determine the safety and efficacy of animal health products. Animal health products generally fall into three categories: veterinary drugs, vaccines or medical feed products, however novel technologies such as monoclonal antibodies may also be developed for the treatment of sick animals. The work carried out under this licence will be designed to meet the requirements of government regulators in Europe and elsewhere, who must agree to the sale and use of these materials in society.

A retrospective assessment of these aims will be due by 19 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The work in this project will be performed to meet the requirements of regulatory authorities responsible for the authorising the marketing of veterinary medicines and medicated feed-stuffs. In doing so, the work enables the development of safe new medicines for use in animals. In addition, the work helps to assure the safety of human consumers who may be exposed to residues of veterinary medicines in the edible tissues and other products of food-producing animals.

As well as assuring the safety of animals and human consumers, the successful conduct of tests will help bring to market materials which improve the health and welfare of animals in which they are used.

What outputs do you think you will see at the end of this project?

Data collected will be information on how animals are affected by potential new veterinary medicines; or how much and for how long the animals retain the medicines in their body; or how much of the medicines are in animal tissues, eggs or milk which might contribute to human food.

Outputs will include simple measures like changes in behaviour, food consumption, growth rate and weight retention or loss. Samples will commonly be taken, particularly of blood, but also other excretions such as urine to assess any changes over time.
Post mortem examination can demonstrate change in function or structure of body organs, including examination at a microscopic level.
The data will be collected to the standards required by government regulators in the UK, Europe and elsewhere, for identifying and excluding inappropriate medicines due to safety concerns, and enabling further development of successful veterinary medicines.

Who or what will benefit from these outputs, and how?

Our clients, typically commercial animal health companies, will benefit from the provision of high quality data. This will help them in their work to produce safer and more effective medicines which can be safely made and used without increasing health risks for animals or for people who could potentially be exposed to them through the eating of animal-based food.
Enabling development of successful veterinary medicines will have a general benefit for animal welfare in society, through diagnosis, treatment or prevention of disease.

How will you look to maximise the outputs of this work?

By conducting the work to the expected quality standard (Good Laboratory Practice) and by following relevant internationally-agreed guidelines, the outputs of the work should be readily accepted in all markets of the world. Collaborations and information exchange with others within the organisation and with our clients, helps to identify and spread information on successful and unsuccessful approaches, and on product development.

Species and numbers of animals expected to be used

- Domestic fowl: No answer provided
- Beagles: 250
- Cattle: 800
- Sheep: 100
- Pigs: 500
- Mice: 150
- Rats: 250

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Most of the animals and life stages used in the project are examples of species which will be given the test items in veterinary practice; the target species. Some laboratory rodents are used to help consider safety of test items which would be given to animals which contribute to human food.
They are also species included in relevant guidance accepted by government regulators in the UK, Europe and elsewhere.

Typically, what will be done to an animal used in your project?

Animals will be given a possible new veterinary medicine or other animal health product, by the same way that this would be done in veterinary practice. Some studies will be to check for any effects of the medicine; some studies will be to measure how much of the medicine is absorbed into the blood, or into the milk, eggs or meat of animal species which contribute to human food. Urine and faeces, and the excreta of birds, are commonly collected, which requires housing the animals singly, although next to each other, in a small cage which allows the urine and faeces to fall through a grid, typically for about a week.
Animals are normally humanely killed after the collection period, and tissues may be taken from the animals post mortem, and analysed.

What are the expected impacts and/or adverse effects for the animals during your project?

The process of dosing animals or taking blood samples may cause minimal discomfort during conduct; no lasting effects are expected.
The medicines are not normally expected to cause any significant harm for the animals.
Confinement for collection of excretions may cause a degree of discomfort and/or reduced activity; a degree of reduced food consumption and/or weight loss may be noted, but this is not expected to be significant based on experience of studies conducted over many years.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

No significant harms are normally identified by examining the animals. This and controlled limits on blood sampling are likely to result in no more than mild severity outcomes in most cases. The single housed confinement of animals for several days, particularly if it includes restricted movement, results in a consideration that this is of moderate severity. This is likely to be required for about half of the animals used in the project, because of the need to collect the excretions of individuals for a sufficient time to complete the scientific aims.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 19 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

While non-animal methods are used in some aspects of the programme of safety assessment of new materials,

they are currently not able to predict effects on whole body systems or to provide information on how much of a material is absorbed, or how animals change and excrete materials. Animal products which contribute to food (meat, milk, eggs) need to be assessed for presence of drug residues which could cause harm to people. This work must be done using the same animals which will be given the medicines, some of which also contribute to human food.
The protocols described in this project are conducted according to internationally-agreed guidelines, to meet the requirements of UK and European law, and are expected to be performed before government authorities will authorise the marketing of new veterinary medicines for animals.

Which non-animal alternatives did you consider for use in this project?

The organisation conducts non-animal tests as part of the many programmes of safety assessment of new materials. However as noted above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes.
A potential *in vitro* approach to comparative metabolism is described in the relevant guideline, and will be considered as and when such studies are requested.

Why were they not suitable?

In vitro comparative metabolism will be used where it is deemed to be an appropriate scientific alternative, and acceptable to government regulators.

A retrospective assessment of replacement will be due by 19 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated upcoming studies.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The animal numbers for studies required by government regulators typically follow those identified in internationally-accepted guidelines, as expected to provide sufficiently significant outcomes.
In situations where there is no definitive guidance on the numbers of animals to be used, the applicant and colleagues will use their extensive experience of related programmes, taking account of statistical significance and scientific advice as necessary, to use sufficient animals for studies to provide robust results.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

All studies are performed according to the principles of Good Laboratory Practice (GLP), which is an

international standard for the quality of experimental study conduct with animals, and is overseen by an independent agency of the government. Following this practice should ensure the quality of studies and acceptance of results by government regulators worldwide, and also reduce the potential for error.
By fully understanding the needs for testing in each case, and by following internationally-agreed guidelines and GLP, the same study should be acceptable to government regulators in all parts of the world where it is needed, removing any need to do the similar work more than once, and use more animals, to meet the needs of all markets where the medicines may be used.
Pilot studies may be used to confirm appropriate elements of study design in advance of full studies, for example, the best timing for taking samples of various body tissues or products to investigate the how much and when a drug is excreted or retained by the animals.
A retrospective assessment of reduction will be due by 19 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Materials are given to the animals by the same way in which they would be given in veterinary practice, most commonly by mouth, in food, or by injection. The dosing methods used are all very well established and common for the animals to be used, and known to cause minimal discomfort based on extensive experience at the site.
Blood sampling is a common need. We follow published guidance on methods and suitable volumes which can be taken while minimising harms to animals.
Restraint or confinement of animals is a common need, to allow collection of samples, generally urine and faeces. Methods used are those with which staff have extensive experience, and the duration of time is minimised wherever possible while allowing completion of the process.

Milk-producing animals are milked regularly using the same processes as are used on farms.

Why can’t you use animals that are less sentient?

The species used are in most cases the same species, and at a stage of life when they would be given the medicines in veterinary practice; and so are the most appropriate scientific models. Where laboratory animals (rodents) are used, they are to help confirm the suitability of study outcomes for people who could potentially be exposed to the medicines by presence in products of animals which contribute to human food. They are the species whose use is expected by government regulators.
The time required to study the outcome of giving the medicines, and for samples to be taken means that continued anaesthesia is impractical, and could interfere with the scientific outcomes.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinement of procedures is an on-going consideration at the site, and new opportunities are particularly

assessed if any concerns are identified during or after studies; for example new methods or additional assessments may be included in future study designs as a result of a review.
Methods to enrich the housing are routinely considered, and changes are commonly introduced to seek to improve the housing. A standing group regularly considers potential refinements at the site, and reports to the animal welfare and ethics review body routinely on this topic.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Where required, blood volume limits are within those proposed in the 2001 publication of Diehl *et al*: A good practice guide to the administration of substances and removal of blood, including routes and volumes.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Both our clients and our colleagues conducting the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which propose refined approaches to conduct of work. **A retrospective assessment of refinement will be due by 19 April 2026**
The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

186. The genetics and therapy of skin disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To investigate skin and related tissues during health, disease and treatments.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Major advances have been made in the understanding of skin diseases including severe birth mark disorders which are currently untreatable. These disorders affect not only the skin but other organs, and often also carry an increased risk of malignancy. To translate these genetic and biological research findings into clinical practice in humans, we must test our novel therapeutic approaches on appropriate mouse models. By doing this we can investigate whether our therapeutic approaches can be delivered successfully, and whether they are both safe and effective, so that they can be considered for clinical trials.

What outputs do you think you will see at the end of this project?

The research is intrinsically translational and focused on generating treatments for untreatable childhood diseases. The final output aim is to identify therapies and/or technologies that will benefit patients directly. There will also be research output in the form of publications, conference presentations and knowledge that will benefit colleagues and other scientists in the field.

Who or what will benefit from these outputs, and how?

Short term benefits (from 2020)

Ongoing participation in patient group events will inform the public of our research, engage them with our work and showcase developments into treating skin disease. We anticipate that early aspects of our research will be published in the coming two years and presented at conferences.

Medium term benefits (from 2023)

Publications and presentations will inform others of our work, assisting and advancing technologies, and forming the basis of further grant applications.

Long term benefits (from 2025)

Assuming successful outcomes, our preclinical research on mouse models will be translated into clinical trials on patients from around 2025. Longer term benefits would be extrapolation of this work to other disorders.

How will you look to maximise the outputs of this work?

This project is already the product of several key collaborations, particularly as regards the delivery systems. Results of the current projects will automatically be shared with the collaborators, allowing them to expand knowledge of their systems and share that knowledge within their respective fields.

Knowledge will therefore be disseminated by our team throughout clinical genetics, dermatology and oncology circles within the medical arena, human molecular genetics and genetic therapy circles in the scientific arena, and delivery systems arenas via our collaborators.

Publication of results will be a priority, as well as dissemination to stakeholder groups in person and using social media and mainstream media outlets. Where practical and possible we will publish unsuccessful approaches

Species and numbers of animals expected to be used

- Mice: 521

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using the mice as a model of skin disease because mouse models are available that have the same genetic mutation that humans with the disease have. This is ideal for progressing with our preclinical investigations of possible therapeutic agents as they will provide a platform to move forward with our research to a practical in vivo system (mice with skin disease causing genetic mutations). Therapeutic interventions on humans with skin disease might in the future be carried out on any postnatal stage. Therefore, all postnatal stages in the mice are of interest to our studies as they will model both early and later aspects of skin disease. In order to best model skin disease in mice, we may also cross any of these strains with other strains with genetic mutations associated with the disease which is not expected (from clinical observation in humans) to have a phenotype at all on its own, but may exacerbate any of the phenotypic features in the strains described here.

Typically, what will be done to an animal used in your project?

Mice with skin disease causing genetic mutations will be generated using standard breeding methods. Baseline phenotyping will involve recording the mouse's phenotypic features and basic non-invasive imaging methods (e.g. dermatoscopy). Where it will be of value, imaging methods requiring general anaesthesia may be carried out and skin or blood samples may be collected for analysis to see if the treatments have worked. Administration of substance will involve application of the substance, most likely to the ear but could be to any part of the body. The application procedure may involve preparation of the skin (e.g. shaving) followed by treatment with the topical cream or ointment. To ensure that the substance remains on the skin for an appropriate amount of time it may be necessary to house the mouse in a single cage for a period after the treatment to prevent other mice grooming away the substance. The mice will also be phenotyped after substance administration in order to investigate the efficacy of the substance for treating the phenotype. All experiments will end with culling of the mice using a schedule 1 procedure.

What are the expected impacts and/or adverse effects for the animals during your project?

Our experiments have been designed to minimise adverse effects on the mice. Phenotyping and sample taking may cause mild, transient pain or distress but this should last no longer than a few seconds. In the majority of imaging procedures, the mice will be under general anaesthetic to minimise distress. Substance administration can potentially cause minor skin irritation, but efforts are made to select candidate substances for their lack of toxicity. On an occasion where the skin is sore as a result of substance administration, local anaesthetic will be used to minimise suffering. As we will be observing the mice regularly following substance administration the mice will only suffer from mild irritation for a short period of time before it is detected. We intend to cull mice before any potential melanoma metastases develop. However, undetected disease may cause pain, weight loss, metastatic tumours and abnormal behaviours. We estimate that this will affect less than 5% of our animals and mice will be culled at the point in which the phenotype is detected.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild (expected of phenotyping / substance administration) = 90% Moderate

(unexpected) < 10%*

*Our goal is to achieve 0% moderate severity. However, we believe due to the nature of our mouse models and investigative nature of substance administration there is a small chance that some mice may experience moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

A mouse model with the precise genetic mutation that causes a skin disease that we are interested in treating in humans is available. This provides an excellent opportunity to study and treat the disease in vivo. Data from these studies will inform us on the effects of the drugs in a living system, with functioning networks (e.g. vasculature, nervous system etc.).

Which non-animal alternatives did you consider for use in this project?

We already and will use in vitro approaches in parallel to our mouse research to inform our experiments. These include experiments on human patient-derived biopsies and cell lines.

Why were they not suitable?

While non-animal alternatives can provide useful data, they limited in that they do not model the complete skin in vivo which is connected to the nervous system, immune system, vascularised, under the influence of endocrinological factors and systemic signalling molecules.

There is often limited availability of patient tissue which prevents the design of experiments as there is little to no knowledge of what will be available for study until the moment of surgery. Even when it is available, it is often variable in nature, from different parts of the body from different patients. This provides variable data with limited opportunity to carry out a carefully controlled, designed study.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may

include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

In order to estimate the number of animals we will use we have carried out power calculations using an online tool. Estimations are listed below. Where possible, these estimations are carried out using what we can learn from published data. In cases where there is not yet published data we will need to carry out pilot studies with a limited number of mice to determine the distribution and difference of data points in two conditions in order to more accurately estimate the number of mice required for an experiment.

Estimation of number of mice required for breeding pairs

This depends on the length of time between experiments, the success of breeding and the numbers they generate. Therefore, assuming we were required to have 10 mice available for breeding at any given time, retiring them every 6 months to avoid detrimental phenotypes. We would use 20 each year. Over the 5 years of the project this would be 100 mice. At any given time we may need to breed both strain 1 and strain 2 mice. Therefore, for the 2 mouse lines we are estimating the maximum number of mice we will need for breeding is 200 (see table 2) Estimation of sample size for studies on strain 1 mice

a) The primary outcome measure will be a decrease in pigmentation

b) The statistical test is a paired one-sided students t-test, with 95% significance at 80% power

c) The effect size considered significant will be a 20% decrease in pigmentation for experiments designed to achieve successful treatment of the phenotype. We account for 10% variability in the results of these experiments between mice. However, until we do preliminary experiments we cannot accurately estimate this.

Using this data with an online calculator the sample size is 8 mice in each group, with two delivery approaches to be tested, with two different concentrations of genetic material. Therefore, the final sample size is 32 mice per experiment. For two experiments and accounting for a 10% attrition rate we estimate we will use 71 mice in protocol 4 (see table 2).
Some mice will be used for baseline characterisation for preliminary studies to establish baseline measurements to direct the therapeutic interventions experiments. We estimate we will require 10 mice in protocol 2 (see table 2)
Estimation of sample size for studies on strain 1 mice

a) The primary outcome measure is the number of melanocytic lesions over the first 6 months

b) The statistical test is a paired two-sided students t-test, with 95% significance at 80% power

c) The effect size considered significant will be a 10% difference in mean number of melanocytic lesions with the baseline estimated to be 3 per mouse, with a variability of 20%

Using this data with an online calculator the sample size is 31 mice in each group, with two variants to be tested, plus a wild-type control group – final sample size is 93 mice in either protocol 2 or protocol 3 (see table 2)
Estimation of sample size for studies on strain 2 mice

a) The primary outcome measure will be a change in pigmentation (also melanoma incidence will be closely monitored).

b) The statistical test is a paired one-sided students t-test, with 95% significance at 80% power

c) The effect size considered significant will be a 20% change in pigmentation for experiments designed to achieve successful treatment of the phenotype. We account for 10% variability in the results of these experiments between mice. However, until we do preliminary experiments we cannot accurately estimate this.

Using this data with an online calculator the sample size is 8 mice in each group, with one control group and two experimental groups, the final sample size is 24 mice. Accounting for a 10% attrition rate we estimate we will use 27 mice in protocol 2 or 3 (see table 2).
Table 2: Estimation of number of mice that will be used

	Protocol 1	Protocol 2	Protocol 3	Protocol 4
Strain 1	100	28	18*	71
Strain 2	100	102	102*	
Total (521)	200	130	120	71

*Protocol 3 will only be used if protocol 2 does not achieve experiment goals

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experiments follow a repeated measures design whereby mice will be treated and phenotyped repeatedly. This will allow us to monitor the effects of substance administration over multiple time points while avoiding the use of multiple groups of mice to carry out these experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To optimise the number of mice in our studies, pilot investigations have been and will be carried out on cell in vitro and skin biopsies ex vivo to determine which approaches are most promising and which are most likely to deliver a successful outcome. This will minimise the number of mice that are needed as fewer investigative experiments will be carried out on the mice whereby little is known about the potential outcome of the therapy being tested.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The animal models that we will use in the project are mice with genetic mutations / phenotypes which model skin disease. These models present with a phenotype which does not cause an aversive effect in the mice. In certain circumstances there will be an increased risk of melanoma but this can be managed by observation and culling of the mice before they reach a stage that causes pain or suffering. In particular, where a carcinogenic substance is used to induce melanoma at a particular location on the skin we are able to monitor this precise location to make sure procedures are carried out before the melanoma causes any aversive effects to the animal.

Phenotyping procedures which may cause distress to the animal because of restraint will be done under general anaesthetic. This will minimise the stress to the animal while allowing us to collect the data as quickly and efficiently as possible. In certain circumstances such as ear biopsies, the temporary restraint and minor transient pain may mean it is less intrusive and stressful to perform the procedure without anaesthesia, or with local anaesthesia instead.

Substance that will be administered to treat the phenotype will be selected for their reported or predicted lower toxicity. This is because the goal of our experiments is ultimately to treat patients and very toxic drugs would be unsuitable. In the event of an adverse reaction the mouse will be treated if this is possible (e.g. local anaesthetic) or culled to prevent worsening of the condition.

Why can't you use animals that are less sentient?

We cannot use animals that are less sentient than mice because:

a) Mouse embryos are not appropriate/accessible for topical application of substances

b) Procedures cannot be solely performed on terminally anaesthetised animals because the effect of the therapy needs to be monitored for some time after application.

c) In general, previous therapies for melanocytic disease including melanoma have been tested on mice before being translated into humans, with mice being considered the least sentient animal model close enough physiologically to humans for this disease. Our efficacy and safety data will therefore be comparable to previous work.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Staff will carry out procedures in an accurate and efficient manner. Given the nature of dermatology research, mice will be monitored for surface abnormalities which will be important for our results and carrying out experiments effectively. This will minimise harms to the animal by identifying phenotypes early that may eventually be harmful to the mouse.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Reference to the topic specific resources on the NC3Rs website will be used to ensure experiments are conducted in the most refined way. This resource provides guidelines and resources on anaesthesia, analgesia,

euthanasia, handling and restraint, humane endpoints, micro sampling and welfare assessment which will be relevant for our project. Experiments will be planned according to PREPARE guidance. Experiments will be conducted with care taken to design and collect information that satisfies ARRIVE (Animal Research: Reporting of In vivo Experiments) guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By subscribing to the NC3Rs newsletter and discussing advances that could be implemented in our research with my research team. Additionally, we will receive communication from named persons and complete annual refresher training.

Home Office

NON-TECHNICAL SUMMARY

187. The gut microbiome's role in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

microbiome, colitis

Animal types	Life stages
Mice	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how the microbes that live inside the gut interact with each other, and with the gut environment. And, to understand how these interactions change between healthy and inflamed conditions.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our guts house trillions of microbes that together make up the gut microbiome. Over recent years, changes to the repertoire of microbes living in the gut, and the balance of numbers between these different types have been linked to a range of diseases with a major impact on global human health. Such diseases include inflammatory disease, infection, cancer, diabetes, obesity and neurodevelopmental disorders.

A better understanding of the gut microbiome could contribute major advances to the diagnosis, prevention and treatment of these diseases. Overall, however, there is still a very limited understanding of how changes to the microbiome come about and what their true roles in disease are. One important reason for this lack of understanding is the limited number of tools available for studying the gut and the microbes living within it. This project aims to address these holes in our understanding by developing new ways to study the gut microbiome.

What outputs do you think you will see at the end of this project?

- The development of new tools and methods to non-invasively study the gut
  The development and testing of new in vitro methods to study the gut microbiome
- The publication of new scientific knowledge about how bacteria in the gut respond to inflammatory diseases.
-

Who or what will benefit from these outputs, and how?

In the medium to long-term, the outputs from this project aim to help people suffering from diseases that are linked to the gut microbiome. The tools we develop and the knowledge we gain through this project may help to develop new types of: diagnostics, to identify disease earlier and with more accuracy; monitoring tools, to help track disease progression and how patients respond to treatment; and treatments for various gut disorders. Because there are many diseases with links to the gut microbiome, our methods, tools, and scientific advances should be of interest for researchers across a range of fields.
In the shorter term, the advances we make throughout this project will provide increased understanding of how bacteria interact inside the gut and how they respond to inflammatory diseases. They will also provide new approaches to study the gut microbiome. These are all of great interest to many researchers, biotechnologists, and clinicians. To maximise the potential reach of our work, we will present our findings at national and international conferences and publish them openly. The tools and data we develop, including advances to in vitro bacterial growth and testing methods that would allow researchers to replace animal experiments with non-animal alternatives will also be shared openly with the research community.

How will you look to maximise the outputs of this work?

Findings will be made available to the broader research community through open-access dissemination, by posting preprints and publishing in open-access journals, and through presentations at scientific conferences and meetings.
All bacterial tools that are developed and raw bacterial sequence data will also be shared openly with the research community through openly accessible strain banks and data repositories so that it can be used in others work.

Species and numbers of animals expected to be used

- Mice: 800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

These studies will be done in mice because this is the lowest vertebrate species that can be used to adequately model the complex nature of the gut and its resident microbes, which is highly dynamic in its microbe-microbe and host-microbe interactions, as well as the gut's response to inflammation. Because the general control of the microbiome and the inflammatory disease response involve system wide responses, we need a model system that can provide this level of complexity.
We also require a model organism that can be maintained without bacteria in the gut so that we can complete experiments in which feed animals a specific set of microbes. So far, mouse models represent the best and most well studied models for both these purposes of bacterial colonisation of the gut and testing inflammatory disease, with several genetic and environmental models replicating a range of the key aspects of human disease
Animals older than 4-weeks old at the start of an experiment will be used to allow for development of the microbiota prior to testing. This is also consistent with the ages of mice studied previously in the published colitis models we will use.

Typically, what will be done to an animal used in your project?

Typically, animals in this project will be fed antibiotics either in their drinking water or by oral gavage. They will then be fed with harmless bacteria by oral gavage. In ~70% of animals our goal is to understand the response of bacteria to the healthy gut and/or to other bacteria in the gut. In ~30% of animals, we aim to understand the response of gut bacteria to inflammatory disease. Gut inflammation will be induced in these animals either by feeding mice with a chemical in their drinking water, feeding mice with a pathogen that causes diarrhoeal disease similar to that in humans, or using genetically altered animals that are susceptible to developing colitis in a similar way to inflammatory bowel disease in humans. In both healthy and inflamed scenarios the response of the gut and the gut microbiome will be monitored by non-invasive methods throughout the experiment - including the collection of faecal samples, the collection of blood samples, or imaging of labelled bacteria in live animals. The latter technique requires short term anaesthesia so that animals do not move during imaging. Most experiments will take between 1 and 4 weeks, although in a small number of experiments animals will continue to be observed for up to 1 year of age so as to study long-term bacterial growth behaviour and to better model

chronic diseases such as inflammatory bowel disease.

What are the expected impacts and/or adverse effects for the animals during your project?

Inflammation:
Animals that are being used in experiments to track inflammatory disease may experience a range of symptoms, which commonly include diarrhoea and weight loss, but may also be accompanied by signs of pain, discomfort and blood present in the faeces. The goal of all of our experiments is to induce the minimal degree and duration of adverse effects necessary to achieve our scientific aims.
Some experiments are designed to test chronic inflammation. Like humans suffering from inflammatory bowel disease, this level of inflammation may cause some discomfort and mild symptoms, but should not cause acute symptoms. Animals being studied for chronic inflammation will typically be studied over the course of 1-2 months, but may be studied up to 1 year old.
Other experiments are designed to test short periods of more acute inflammation, such as are experienced during pathogenic infections in humans. These experiments will usually involve acute inflammatory activation for around 1 week. Mice will be monitored particularly closely throughout any period of expected acute inflammation, and any animals that do show signs considerable weight loss or pain for more than 24 hours will either be humanely killed by a Schedule 1 method, or the induction of inflammation will be removed to allow recovery, thus limiting their suffering.
Imaging:
Some animals will be put under brief anaesthesia (less than 15 minutes), so that they stay still while we image the bacteria in their guts. They may also have an injection at this time to introduce dyes that help us to see more of their bodies. They are expected to recover quickly and will be given care following the anaesthesia if needed, just like a human would be.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect that ~70% of animals will experience only mild severity procedures, and ~30% will experience moderate procedures including inflammatory induction and short-term anaesthesia for non-invasive imaging studies.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are needed, because no alternative model currently incorporates all elements of the mammalian gut environment, including having different spatial regions that favour different bacterial species, immune responses and gradients of oxygen. These factors are particularly important for the goal of understanding the response of bacteria to the gut environment and to each other during inflammatory disease, which is known to involve changes to each of these elements. They are also important for our studies of how bacterial behaviour varies in different parts of the gut.

Which non-animal alternatives did you consider for use in this project?

Some experiments in this project fall within a broader research programme that aims to develop *in vitro* bacterial culture methods that can sufficiently reflect critical aspects of the gut microbiome in its native environment to allow for replacement of animal use for similar experiments in the future. An important factor in developing such a platform, however, is benchmarking of results against the *in vivo* system.
In addition to culture methods, we also considered the use of *in vitro* co-culture of bacteria with mammalian tissue, the 'gut-on-a-chip' culture system, and the use of human tissue or faecal samples.
Why were they not suitable?

In vitro co-culture of bacteria with mammalian tissue in general cannot be used to study long-term interactions due to the incompatibility of the conditions created for and by bacteria in culture and the conditions needed for mammalian cells to stay alive. Mammalian cells need oxygen and low acidity. Anaerobic bacteria cannot be exposed to oxygen and often make the media acidic. In in vitro cultures, mammalian cells generally don't produce the protective mucous layers that they would in the body, which provides an important barrier preventing direct interactions between bacteria and the cell. Without mucous present bacteria often have a direct toxic effect on the mammalian cells.
The most advanced examples of 'gut-on-a-chip' technology have shown co-culture of complex set of microbes including anaerobic bacteria for up to 5 days. However, they still have not replicated key aspects of the mammalian gut inflammatory environment including immune system responses and spatial community dynamics, thus making them inappropriate for our purposes.
Because we need to use controlled experimental conditions, and we need to administer specific bacterial strains our experiments also cannot be performed with human faecal or tissue samples.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

All experiments will be designed so that the minimum number of animals necessary will be used. In many cases, our experiments have been designed to perform an initial screen from a pooled set of samples followed by individual validation of top performing candidates. The estimated numbers are calculated assuming typically the top 5 candidates will be tested, along with relevant controls, however we will only individually test strongly performing candidates so this number may be reduced should less high quality candidates be identified.
Due to the potential for bacteria to be shared between co-housed animals the experimental unit has been defined as each individual cage initially and the numbers assume that mice will be socially housed for longer term experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experimental design is based on a combination of experience, consultation with published studies and experts in the field, and resources available on the NC3Rs website. Where possible, we will follow repeated measure experimental designs to allow animals to act as their own paired controls pre- and post- colitis induction. This

measure reduces animal numbers both by limiting the need for two different groups of animals, as well as reducing inter animal sources of variability.
While the experimental unit will initially be defined as a cage of animals, for experiments lasting less than one week we will individually house animals to reduce the number of animals needed. In addition, we will analyse results from co-housed mice throughout the study and should we find evidence that mouse-to-mouse transfer does not affect results in our experimental systems any subsequent experiments will instead use an individual mouse as the experimental unit. In some cases this could halve the number of animals used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The proposal outlines the use of established models of bacterial colonisation and colitis induction that require minimal optimisation. Where possible we will also use methods available to reduce variation between animals. Together these will reduce the number of animals required for statistical significance.
Where relevant, we will use female mice to allow for mixing of animals between cages prior to experiments. This aims to reduce cage-to-cage differences in the microbiome because mice share their microbes by eating each other's faeces. By reducing variation between cages this in turn reduces the number of animals and repeat experiments required.
For new analysis techniques we will use pilot studies on either unused tissues from collaborators or small numbers of mice, to reduce animal usage and ensure that measurement techniques will achieve their scientific aims before using them on larger numbers of animals.
Several experiments will also begin with screening studies, either in vitro or in vivo, which we have used to allow for testing of hundreds to thousands of bacterial strains in a single experiment. Only those bacterial strains that show the highest promise from screening studies will then be followed up individually in animals. Where possible we will use the screening data to calculate group sizes in these follow up experiments to use the minimum number of animals for our scientific goals. This strategy considerably reduces the number of animals needed to achieve the aims of the project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use well established models for colitis that replicate critical aspects of human diarrhoeal disease and inflammatory bowel disease. Our humane endpoints are in place to ensure that ongoing pain, suffering, distress or lasting harm is minimised.

Why can't you use animals that are less sentient?

Non-mammalian animals are not suitable for use this work as the required immune systems, physiological conditions and spatial variations in the gut that favour specific species do not exist in lower species. The

long-term nature of the experiments, from days to months, makes terminal anaestheisia inappropriate.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Dosage of inducers will be chosen with the aim of triggering colitis that is not severe enough to trigger our humane endpoints. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All experimental procedures will follow LASA guidelines. Experiments will be designed and undertaken in accordance with the PREPARE guidelines and communicated in accordance with the ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed about advances in the 3Rs though the NC3Rs website, and through monitoring up to date science in this area through regular literature, conference attendance and discussions with colleagues. Advances that would advance any of the 3Rs will be implemented throughout the project where possible.

Home Office

NON-TECHNICAL SUMMARY

188. The Immune Response to Tissue Repair and Cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

inflammation, wound healing, cancer, scarring, live imaging

Animal types	Life stages
Mice	adult, juvenile
Zebra fish	adult, neonate, embryo, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To advance understanding of the role inflammation plays in tissue healing and cancer progression with the aim of identifying interventions to improve the outcome of the repair process for traumatic injuries, surgery and cancer treatments.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Scarring following injury is a major medical problem. In addition to the visible disfigurement of skin scars, scarring also results in a loss of tissue function that can restrict movement and can have adverse psychological effects. Scarring occurs as a result of the laying down of extra-cellular matrix, such as collagen, at the injury site. It is well recognised that while inflammation is pivotal in some aspects of healing, it also plays a major role in determining the extent of tissue scaring; however, the precise mechanisms that drive this process remains unclear. The outlined work aims to advance fundamental understanding of the processes involved in tissue repair and the associated inflammatory response and thereby identify interventions that could be used to improve the outcome of the healing process. It is also clear that inflammation plays a role in cancer initiation and progression in ways that are very related to the wound inflammatory response. Consequently, these studies will also investigate aspects of cancer inflammation in order to identify interventions for improving cancer therapies, particularly those therapies, such as surgery and radiotherapy, that trigger a wound inflammatory response in tissues.

What outputs do you think you will see at the end of this project?

The key output of these studies will be to advance understanding of the mechanisms underpinning normal healing of skin and other tissues and to identify how these alter when healing fails e.g. as in a chronic, non-healing wound, or in scenarios where major scarring occurs. The study will identify some of the key genes and signalling pathways involved in wound healing, in particular, those associated with the wound inflammatory response, and will evaluate if modifying these can improve the healing process e.g. by either speeding it up or reducing the level of tissue scaring. The study will also identify key genes and signalling pathways involved in the inflammatory response to cancer and determine the importance of these in influencing the progression or retardation of the cancer. In particular, we expect to determine the influence that the inflammatory response initiated by clinical interventions used in the treatment of cancer (e.g. biopsy, surgery or radiotherapy) has on the progression of the cancer and whether modulating this could improve outcomes. For each arm of this research our goal will be to publish our findings in top ranking, open access journals.

Who or what will benefit from these outputs, and how?

In the short term, the findings of our study will benefit scientists working in the field of wound healing and cancer treatment. In the medium term, the findings are expected to benefit biotech companies/big pharma developing new products and interventions to minimise scarring and improve would healing. In the long term, the findings are expected to benefit clinicians and patients through the development of more effective treatments and interventions to improve the outcome of tissue repair and cancer treatments.

How will you look to maximise the outputs of this work?

To maximise the outputs and the impact of our research we will collaborate, as we already do extensively, with clinicians specialising in plastic surgery and cancer therapy. In so doing, our research directions are guided by real and relevant clinical problems. In addition, these collaborations facilitate the rapid translation of our findings into the clinical setting. The findings of our research will be widely disseminated through presentations at international scientific meetings and by publications in high impact, open access journals and review articles.

Species and numbers of animals expected to be used

- Mice: 2500
- Zebra fish: 57500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Our work will adopt an integrated approach utilising in vitro and in vivo models. With respect to the latter, our studies will utilise the fruit fly, Drosophila melanogaster (not HO regulated), and zebrafish and mice to investigate aspects of inflammation following wounding and cancer initiation.
In zebrafish we do our work wherever possible at larval stages because these are translucent and enable live imaging studies. Some studies progress to adult fish, in particular where we wish to study later stages of cancer progression and/or the role of the adaptive immune system which is not present at larval stages. Our mouse studies are only of the wound inflammatory response (not cancer studies) and are largely performed on adult animals where we can study how inflammation impacts all of the cell lineages of the repair process (wound fibroblasts, endothelial cells of vessels etc). On occasions we also study neonatal mice where it is useful for there to be no hair, or if the KO line is not healthy at later stages.

Typically, what will be done to an animal used in your project?

The majority of the animals we require are only used for creating and maintaining lines i.e. for breeding. For our fish studies these are often of reporter lines where, for example, specific cells are labelled with a fluorescent marker. For our mouse studies the breeding will be of heterozygous mice to generate litters where some of the progeny are -/- KO mice.

A relatively small number of our fish (approximately 10%) are required for wound healing and cancer experiments; much of our data can be collected from 5dpf (non-regulated) larvae. Of those progressed to wound healing studies, the majority of those beyond 5days post fertilisation will be anaesthetised and receive a small single wound produced using either with a laser or needle; they will subsequently be either immediately live imaged (for up to several hours) while still under anaesthetic, or allowed to recover from anaesthesia and subsequently imaging on a number of occasions (not more than once daily) under anaesthesia before being killed. Generally they will only be imaged once but on occasions we will image the same fish up to 6 times, over 2 weeks. Those fish used to model various cancers will be anaesthetised at various timepoints during cancer progression and imaged as above. A small number of fish with cancer will receive a localised lesion, using a laser or needle, close to the cancer or be given radiation treatment in order to replicate routine procedures conducted on cancer patients so as to study how these influences the progression of the cancer.

For wound healing studies in mice: the mice will be anaesthetised, the wound site cleared of hair, and up to 4 small full depth punch biopsies made through the skin before being allowed to recover form anaesthesia. Subsequently, the mice will be killed at predetermined time points and tissue collected for analysis.
What are the expected impacts and/or adverse effects for the animals during your project?

The zebrafish used in wound healing studies are not expected to show any adverse signs, and are expected to

continue to swim, feed and interact with other fish completely normally. The fish used in the cancer studies are expected to grow into adulthood and to continue to swim, feed and interact with other fish completely normally whilst carrying the tumour. All fish are carefully monitored and any showing any abnormal behaviour are promptly killed.

Following recovery from anaesthesia post wounding, mice are expected to immediately resume normal behaviour and to appear unperturbed by the skin injury. The wound created scab over within 24 hours and are usually fully healed over within 7 days. All mice are carefully monitored and any showing abnormal behaviour are promptly killed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of animals (~78% of fish and 80% of mice) will only be used for breeding in mild severity protocols; another 12% of all fish will be used in breeding/line generation protocols that could be up to moderate. Animals entering the wound healing protocols (~7.5% of fish and 20% of mice) will undergo general anaesthesia and a small surgical injury will be made to superficial tissues. Upon recovery from anaesthesia these animals are expected to immediately resume normal behaviour and to show no outward signs of suffering, nevertheless since a surgical procedure has been performed a moderate severity level has been allocated to these studies. Only about 2.5% of fish will be used for cancer studies. The fish are not expected to show outward signs of suffering as a result of the cancers. However, as the study involves imaging under anaesthesia, and in a small number of cases wounding of the tissues close to the cancer under anaesthesia, a moderate severity level has been allocated to these animals.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of vertebrate animal models is crucial to studies of the wound inflammatory response and its influences on other cell lineages since these processes are far too complex and involve too many individual and interacting cell lineages (ie several different immune cell lineages, fibroblasts endothelial cells, pericytes, adipocytes) to be effectively modelled using cell culture, computer simulation, or non-vertebrate animal models such as the fruit fly (see below). For these reasons, and because ultimately we want our experiments to be clinically relevant, at least some of our experiments must be performed in vertebrate species such as the zebrafish and mouse. Nevertheless, to minimise animal numbers, as far as possible zebrafish studies will be performed during the embryonic larval stages of development.

Which non-animal alternatives did you consider for use in this project?

Some aspects of tissue repair can be modelled to a certain extent by various in vitro experiments. The most basic of these is the "scratch wound" assay which is performed on a layer of cultured cells. Where feasible we will use the “scratch wounds model” for preliminary studies of re-epithelialisation. In addition, we will also use a modified version of the "scratch wound" assay involving co-cultured cells for biochemical investigation of how

two wound cell lineages interact with one another (e.g.
macrophages and endothelial cells). Our lab has also pioneered the use of the fruit fly (Drosophila) as a model of wound healing and its associated wound inflammatory response and we have published several Drosophila papers addressing the genetics and cell biology of these processes during the period of our last PPL. Nevertheless, to validate the clinical relevance of findings made in cell culture and fruit flies will always require the use of vertebrates models and, in particular, a mammal. However, wherever possible, zebrafish studies will be performed during the embryonic larval (nonregulated) stages of development.

Why were they not suitable?

In vitro studies can never completely replicate the complex interactions that occur in vivo and many aspects of wound and cancer inflammation can't be fully modelled in flies; for example, Drosophila have only one "leukocyte-like" lineage (the hemocyte), and have no real equivalents to the fibroblasts or melanocytes of mammalian skin, and so we are unable assess how these cells are influenced by wound inflammation in this model. Consequently, whilst we use cell culture and fruit fly studies to complement our studies of vertebrate repair and cancer, they cannot completely replace them.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals requested is based upon our estimate of the likely number of genes and cell types that will require manipulation in order to meet our objectives. In estimating the group size for the studies we have undertaken extensive analysis of data from our previous studies as well as drawing on data published by others and undertaken power calculations, with the help of G*power software. These calculations have been further checked by a specialist bio-statistician. The vast majority of animals requested will not be used in experimental studies but are required in order to breed animals with the genetic make-up needed for the experimental study. Here again we have drawn on our own extensive experience and published information in estimating the number needed.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have and will continue to use the Experimental Design Assistant (EDA) from NC3R, to plan all experiments. We are also constantly alert to the possibility that effect size differences for new assays will be greater than we expect and mean we can reduce n's and retain power to detect significance between experimental groups. Prior to undertaking animal studies, preliminary investigations using our established cell culture and fruit fly models will be used, wherever possible, to test developing hypothesis and to assess the safety of novel compounds.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The vast majority of animals used are needed to create more animals with the correct genetic make-up for experimental studies. Care will be taken to ensure that the breeding programmes is undertaken with optimal efficiency. Wherever possible, and utilised a lot during our last PPL period, we will coordinate breeding with colleagues working in related fields to maximise the usage of the animal produced e.g. by sharing clutches of transgenic fish amongst several researchers all using the same line/cross.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use both mice and zebrafish to model the wound inflammatory response, but only zebrafish to investigate the cancer inflammatory response. All procedures that could cause pain, suffering or distress to the animal will be performed under general anaesthesia. Upon recovery from anaesthesia the animals will be carefully monitored and any showing more than transient signs of pain or distress will be killed. The use of zebrafish enables us to minimise the numbers of mice needed but unfortunately, not all the complexities of mammalian skin healing and inflammation will be perfectly modelled in a fish and so we need to continue some studies in mice.

Why can’t you use animals that are less sentient?

A large proportion of our studies will be conducted using zebrafish larvae; however, to generate these still requires the use of adult fish (for breeding). We also use Drosophila as a wound inflammation model but due to some fundamental differences in their immune system (e.g., only one “catch all” lineage of immune cell (rather than several lineages of innate and adaptive immune cells in vertebrates), and no known chemokines), we must use vertebrate models to investigate some of the complexities of the inflammatory response to tissue repair and cancer. Wherever possible zebrafish will be used in preference to mice for our studies; however, not all of the complexities of mammalian skin healing and inflammation will be perfectly modelled in a fish and so we need to continue some studies in mice, where validation of a mechanism in mammalian tissues will make it more likely for our findings to be of clinical relevance.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All procedures that could cause the animals pain, suffering or distress will be conducted under general anaesthesia. Upon recovery from anaesthesia any animal that show more than transient signs of pain will be killed. in addition. we will be guided by colleagues within our scientific community (and beyond) with regards to best 3Rs practice for the procedures being undertaken. In particular, because zebrafish is a relatively new animal mode, we are continually hearing from colleagues at international meetings about innovative ways for minimising "harms" to the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All surgical procedures will be conducted in line with LASA recommendations. For our mouse studies we are constantly keeping up-to-date with published studies on, for example, optimal anaesthesia etc. Our zebrafish husbandry is in line with that recommended by FELASA.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We have a continuing dialogue with NC3Rs team; indeed I have talked at several NCR3s conferences in recent years about the use of flies and fish as substitutes for the use of rodent models. In our lab we constantly strive to develop models that might complement/partially substitute for investigations normally only possible in mammalian studies. For example, in one recent published study, during the period of our last PPL, we showed that Drosophila, despite their largely "open" circulatory system, can be used to investigate the rate limiting step in inflammation of extravasation of immune cells from vessels to wounds.

Home Office

NON-TECHNICAL SUMMARY

189. The immunobiology of mycobacterial infection

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Tuberculosis, mycobacteria, vaccination, therapy, pathogenesis

Animal types	Life stages
Mice	adult, pregnant, juvenile, neonate, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall goal of this project is to investigate the interaction between the bacterial pathogen, *Mycobacterium tuberculosis*, and its vertebrate host with the aim of finding better ways to treat and prevent Tuberculosis. The work aims to develop new antimicrobial treatments and therapies that promote the host response, thus improving treatment outcomes and tackling antimicrobial resistance. We will also develop tools to find and test novel Tuberculosis vaccines.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Tuberculosis is a major threat to global health with 9 to 10 million new cases annually and 1.5 million lives lost every year. In addition, the World Health Organisation estimate that 2 billion people carry a latent or dormant *Mycobacterium tuberculosis* infection and thus provide a reservoir of potential infection that will be with us for many years to come.

Current drugs are effective in treating infections, but this takes 6-months leading to poor patient compliance and an increase in drug resistant infections. In fact, about 29% of all deaths caused by antimicrobial infections today are due to drug-resistant TB, making it a priority to development of new drugs and therapies that work faster, more efficiently and can treat drug resistant infection.

The current tuberculosis vaccine (BCG) prevents childhood disease but is ineffective against adult tuberculosis in the countries that bear the highest burden of infection. There is an urgent need for new vaccines to prevent active disease, as well as to prevent reactivation of latent infection.

The aim of our research is to understand the biology of tuberculosis in terms of the interaction between host and pathogen with a view to developing improved TB control strategies.

What outputs do you think you will see at the end of this project?

Outputs will include new information on the role those various mycobacterial genes play in the infection process and whether they are suitable as intervention points for new drugs, vaccines or immunotherapies. This information will be disseminated by publications in the scientific literature.

Outputs will include validation of a novel model for vaccine testing that uses fluorescent mycobacteria to report on how well the vaccine works, and validation of ways to deliver drugs to the infected lung. This information will be disseminated by publication in the scientific literature and will form part of the data required to take some of this work into human clinical trials.

Who or what will benefit from these outputs, and how?

In the short 5-year term the outputs will benefit the research community by establishing the suitability (or not) of specific genes as targets for drugs, vaccines or immunotherapy.
In the longer-term suitable lead compounds, fluorescent reporter models and lung delivery systems may be taken on by companies in the Bioscience sector, and ultimately lead to patient benefits.

How will you look to maximise the outputs of this work?

Findings will be made available to other scientists through publication in open-access journals and presentations at scientific conferences and meetings. Tissues harvested at post-mortem are stored and made available to other researchers.

Species and numbers of animals expected to be used

- Mice: 2,500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The interactions between bacteria and the infected human are very complex and for now we need to use animals to study the progress of infection and how best to either prevent it with a vaccine or treat it with a drug. Current non-animal alternatives cannot reproduce all of the complexity of this system. The inbred mouse model used here is well characterised and clinical signs of infection are readily recognisable; it also causes the least overall harm compared to other animal models of tuberculosis whilst still achieving the objectives.

Typically, what will be done to an animal used in your project?

In a typical experiment to test a new vaccine for tuberculosis, two groups of animals will be vaccinated by an appropriate route and rested for 4 weeks; another two groups will receive a sham vaccine as control.

One group of vaccinated and one group of unvaccinated animals are then given an dose of fluorescent reporter mycobacteria in the skin to test how well the vaccine works. The fluorescent signal from the bacteria in the skin will then be photographed three times a week with a modified camera over 3 weeks to measure the loss of the signal over time as the bacteria are killed by the vaccine immune response.

Other groups of vaccinated and unvaccinated animals are given an infected by the intranasal route with virulent mycobacteria. Animals are humanely culled around 4 weeks after infection and the number of bacteria in the lungs and spleen are counted and compared between vaccinated and non-vaccinated. Tissue samples are collected for immunological analyses.

What are the expected impacts and/or adverse effects for the animals during your project?

None of the procedures used should cause lasting harm, and anaesthetics are used when the procedure may cause stress. Infection with wild type virulent mycobacteria eventually leads to a pneumonia-like illness with respiratory distress and weight loss. Any animal that shows such deviation from normal health (piloerection, hunched posture, abnormal gait, respiratory distress, inactivity or inappetence) will be monitored more frequently and supportive treatment provided such as warming and wet mash. Should the signs persist for a period of 24 hours the animal will be humanely killed. In addition, any animal that loses 20% of its starting body weight will be culled.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Around 50% of animals will experience mild severity, with the remainder moderate.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The interactions between bacteria and the infected human are very complex and for now we need to use animals to study the progress of an infection and how best to either prevent it with a vaccine or treat it with a drug. Current non-animal alternatives cannot reproduce all of the complexity in this system. The inbred mouse model used here is well characterised and clinical signs of infection are readily recognisable; it also causes the least overall harm compared to other animal models of tuberculosis whilst still achieving the objectives.

Which non-animal alternatives did you consider for use in this project?

We make extensive use of laboratory cell culture models to characterise as many aspects of the infection process as possible. Novel cell culture systems are being developed with combinations of cells, which we are planning to test. Models of *in vitro* granulomas and *Galleria mellonella* larvae are available to monitor the early stages of infection and the factors involved; where possible these models will be used as a replacement. Part of this project will be the development of a mycobacterial system that can be used in humans to rapidly test novel vaccines and drugs.

Why were they not suitable?

The *in vitro* models are useful for preliminary screening of therapeutic agents but cannot replicate the immune response need for vaccine evaluation. The *Galleria mellonella* model is similarly useful as a screening tool for therapeutic agents, but its internal anatomy and physiology are distinct from mammals. Thus, therapeutic agents ultimately need to be tested in animal models of infection so that effects like tissue absorption and penetration, agent stability and half-life can be assessed.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals is estimated based on previous experience of the number and type of experiments we will do over the duration of the project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where possible we will compare different treatments or regimens in the same experiment so that control mice are shared, thereby reducing the overall number of animals. Experiments are repeated for scientific validation and to ensure reproducibility of the results.

Mice are marked to allow individual analysis. Each group of mice is randomly assigned to an experimental or control group and, where appropriate, analysis is blinded to prevent bias.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We have developed bioluminescence and fluorescence imaging tools for mycobacteria that allow non-invasive detection of bacteria within animals in real-time. The combination of imaging and monitoring weight loss results in a reduction in the numbers of animals used during experiments and the level of suffering, by the implementation of more humane endpoints. We routinely collect and store fixed tissues from infected animals that are shared to with collaborators in the field.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse model causes the least overall harm compared to guinea pigs, rabbits and non-human primates, which experience more severe disease. Inbred mice are widely accepted as models for human infectious diseases; they are genetically identical which reduces the number of animals required, compared with outbred animals, where the genetic makeup varies unpredictably. Mice will be infected with mycobacteria through the nose, and some treatments are given via the trachea into the lungs. Both of these procedures are performed under anaesthesia to limit stress, and neither cause lasting harm, although mice may eventually develop symptoms due to the infection.
Why can’t you use animals that are less sentient?

As we aim to model human infection, we need to use adult mice, as a model. Infection and symptoms are followed over time, so live animals are required.

The *Galleria mellonella* insect larvae model is used for screening but has a primitive immune system and anatomy and is not suitable for vaccination or therapeutic testing.

The natural infection of zebra fish larvae with *Mycobacterium marinum* has been used in a number of studies to investigate the interaction between the bacteria and cells of the innate immune response, with some studies using adult fish in which the adaptive immune response has also developed. However, *M. marinum* does not cause human tuberculosis and these microorganisms are genetically quite different from *M. tuberculosis*. This means the zebra fish-marinum model is of limited use for evaluating the role of specific *M. tuberculosis* genes in pathogenesis, or for vaccination or therapeutic studies.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Daily monitoring of infected animals, with monitoring of body weight allows us to define more humane endpoints. Pain management will be provided on the advice of the Veterinary Surgeon for any procedure or situation that requires it. Training is continual making use of new developments and technologies as they come available.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow published guidelines issued by the *Laboratory Animal Science Association*, the *National Centre for the Replacement, Refinement and Reduction of Animals in Research*, and the RSPCA.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The scientific literature and conferences are sources of information on new developments and technologies, as are workshops run internally on the Replacement, Refinement and Reduction of animal use. Our previous work has developed and implemented imaging tools for use in infection models, and the reporter systems we developed have been widely distributed.

Home Office

NON-TECHNICAL SUMMARY

190. The role of genes in blood vessel formation

Project duration

3 years 0 months

Project purpose

- (a) Basic research **Key**

words

blood vessels, development, Protocadherin 1

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to assess the role of genes in the formation of blood vessels. Blood vessel formation is an essential process in development of organisms and it also plays a pivotal role in a number of diseases including cancer and inflammatory disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to undertake this work because we are not able to model all aspects of blood vessel formation in vitro. This is because there is a complex interaction between a number of different cell types which drives this process. It is known that vessel formation is strongly driven by low levels of oxygen. Then as new vessels form and blood flows, there is increased oxygenation of tissues terminating the signals for this process. Modelling these dynamic changes in oxygen and the element of blood flow is not yet possible in vitro. Understanding how blood vessels form has the potential to yield novel therapeutic interventions for diseases where vessel formation contributes to the pathology of the disease; this included solid tumours, chronic inflammatory diseases such as rheumatoid arthritis and eye diseases including diabetic retinopathy and age related macular degeneration. There are also diseases, including ischaemic heart disease, where a better understanding of vessel formation, may enable the development of therapies that will drive vessel development to restore blood flow to damaged tissue.

What outputs do you think you will see at the end of this project?

At the end of this project we will generate new information about the function of PCDH1 and whether this gene products plays a role vessel formation both during development and in the adult. Information will be disseminated in the form of conference presentations and publications. Indications that PCDH1 does regulate vessel formation could yield novel therapeutic strategies for manipulating vessel formations in diseases such as cancer where this process contributes to the pathology. Thus, positive results could generate further funding to explore these options.

Who or what will benefit from these outputs, and how?

In the short term this project will yield novel information about the role of PCDH1 in vessel development, this has not been investigated and so our findings will be of benefit to the scientific community. They will gain a greater understanding of the function of this gene and gain greater insight into the role of this family of genes as a whole. In the longer term, if we discover a role for PCDH1 in vessel development, this will yield the potential for future funding to investigate ways of modulating PCDH1 function to regulate the process of vessel formation, This could have impacts on diseases such as cancer, since solid tumours depend on vessel formation for their growth and metastasis. In addition, discovering new ways to promote vessel formation could impact upon the treatment of heart attack or peripheral artery disease where promotion of new vessel growth would be beneficial.

How will you look to maximise the outputs of this work?

We will publish our work in open-access peer-reviewed journals in accordance with the ARRIVE guidelines and present at conferences to disseminate new knowledge about the function of this gene.

Any results, including negative data, about the extent to which loss of PCDH1 affects vessel formation will be reported. We collaborate with a group external to our establishment on this gene and our results will be of benefit to them. We will also share our findings with groups internal to the establishment who have an interest in some of the proteins with which PCDH1 interacts.

Species and numbers of animals expected to be used

- Mice: 200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice as the process of vessel formation has been well studied in this species and closely resembles that found in humans. In addition, the technology of manipulating the genome is well developed in mice enabling us to investigate mice lacking our gene of interest in either all cells or cells of a particular type. The tissue distribution of our gene of interest PCDH1 in mouse very closely resembles that of human PCDH1; this not the case for lower organisms such as zebrafish which can be used to study the function of some genes in vessel formation.

Because we are investigating developmental blood vessel formation we will evaluate the vascularisation of embryos and also in the retina, where vessels develop shortly after birth. To study blood vessel formation in adult mice, we will also use mice at this stage of life.

Typically, what will be done to an animal used in your project?

The project involves breeding mice which lack PCDH1 in either all cells or just endothelial cells, the cells that line blood vessels. We will then examine blood vessel development at various stages of development in mice after they have been humanely killed. Of particular interest will be to examine vessel development in embryos just prior to mid-gestation and the vessels of the retina examined shortly after birth. We will also investigate vessel formation in adult mice in two contexts. In the first we will examine vessels that develop in tumours. To investigate this, tumour cells are implanted under the skin into the flanks of mice. The size of the tumours, which take about 3 weeks to grow, will be monitored using callipers (that provides precise measurements in millimetres). Once the tumours have reached their size limit the experiment will be ended and the mice killed, as well as measuring the rate at which the tumours grow the tumours will be examined for various parameters of vessel formation and functionality. We will also use a second method to investigate vessel formation in adult mice, this is driven by different growth factors more associated with wound healing, in this model small pieces of sponge are implanted under the skin under anaesthetic, these sponges are then injected with factors to promote vessel development every 2-3 days for two weeks. The mice are then humanely killed and the sponges are removed and examined for vessel growth.

What are the expected impacts and/or adverse effects for the animals during your project?

We do not expect the breeding to be associated with adverse impacts, reports from our collaborators suggest that mice lacking PCDH1 are smaller than those that do not lack PCDH1 but are otherwise healthy.

Mice implanted with tumour cells will experience tumour growth which will not cause any pain. When the tumour is become larger, they can impede the normal behaviour of the mouse. Rarely the skin around the tumours may ulcerate. Animals will be killed according to monitoring for adverse effects set out in the protocols.

Mice implanted with sponge will suffer discomfort immediately after the implantation of the sponge, however analgesics are administered to minimise the pain. After this has healed the mice tolerate the implanted sponge well and the injections into the sponge are done while the mice are lightly anaesthetised and this does not result in adverse effects. Rare adverse effects associated with poor wound closure and infection can occur and if they do animals will be killed according the monitoring for this as set out in the protocol.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The breeding protocol is associated with a moderate severity, this is because mice lacking PCDH1 in all cells are smaller and there is a greater pre-weaning loss of these mice. Though smaller they do not show any signs of ill health. We expect to see this severity in around 25% mice.

The tumour implantation protocol has a moderate severity expected in 90% of the animals, it is probable that in 10% of the animals the tumours may not grow properly.

The sponge implantation protocol has a moderate severity in all animals, this is because the severity is associated with surgical implantation of the sponge which occurs in all animals undergoing this protocol. Once implanted the sponge does not change in size.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We use a number of non-animal systems which replicate limited aspects of vessel formation to investigate genes of interest before considering the use of animals to more fully explore the functions

of these genes. Animals are needed to assess blood vessel formation because at the moment there are no laboratory models that can fully replicate this process. This is because it is not yet possible to incorporate the different types of cells, signals and the aspect of blood flow that are all involved in driving vessel development.

Which non-animal alternatives did you consider for use in this project?

We have explored a number of experimental systems that replicate aspects of blood vessel formation. The models that best replicate this process involves co-culturing endothelial cells, which are the cells which form new vessels, with other cell types that can provide supporting signals and scaffold. The Chick embryo CAM chorioallantoic membrane (CAM) model is another method that can be used to investigate blood vessel formation.

Why were they not suitable?

Our co-culture model promotes the formation of endothelial tubes, but these do not fully resemble vessels found in the animal and the aspect of blood flow is missing. It is not yet possible to fully replicate the conditions found in the animal which cause blood vessels to be formed. We are constantly reviewing and exploring new non-animal systems to try to better model vessel development. We are currently investigating the possibility of using stem cells which can be differentiated into endothelial cells and used in organoid systems which support the formation of vessel like structures. While these models show promise they do not yet fully replicate the process of vessel formation as it occurs in animals. While the chick embryo CAM model has been very useful in testing the angiogenic properties of various factors, it would not be suitable for investigating PCDH1 because we are not able to undertake genomic modifications in the chicken and expression of PCDH1 in chicken has not been reported to have a vascular expression pattern.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers have been estimated based on our previous experience with these experimental models combined with the use of statistical tools (power analyses) to determine the numbers of mice required to give meaningful data. Given that our gene of interest has not been previously studied in the context of vessel formation, effect size will be determined small pilot experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The NC3Rs experimental design tool and its associated statistical analysis tool has been used to assist with experimental design and reduce the number of animals used. We will use methods to reduce

subjective bias, and maximise the information obtained from a minimal number of animals by, for example, longitudinal studies monitoring tumours as they grow.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use the most efficient breeding strategies to generate mice of the correct phenoytpe and monitor breeding closely so as not to generate excess animals. All experiments will be designed in line with the PREPARE guidelines checklist to ensure the best possible design. In addition, data will be published in Open Access Journals and in accordance with the ARRIVE guidelines. Negative data, should it be generated will also be reported.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The angiogenesis assay we propose in this application is the rodent subcutaneous sponge model. This model is robust, sensitive and reliable with the result that we use a minimum number of animals for each experiment. This model is the most refined being far less invasive –than other angiogenesis assays typically used such as the rabbit corneal assay, skin flap assays or the dorsal air sac assay. The subcutaneous implantation model of tumour growth is one of the most refined tumour models combining ease of implantation by subcutaneous injection and facile monitoring of tumour growth.

Why can’t you use animals that are less sentient?

Previous research by many groups has documented that the mouse is the lowest organism with sufficiently similar angiogenic patterns to man to allow meaningful evaluation of mechanisms of blood vessel formation. It is possible to use vessel development in zebrafish embryos to determine the role target genes may play in developmental angiogenesis. However, use of this model is contingent on the genes of interest having zebrafish orthologues with similar expression patterns to their mammalian counterparts. The gene of interest in this study has been duplicated in the zebrafish genome and has an altered expression pattern in this species. By contrast, mouse and human show similar expression patterns of the gene being studied in this licence application. We will evaluate vessel development in mouse embryos from day 9.5 – 13, during which time the early vessels and first angiogenic vessels are formed, and before embryos are covered by ASPA.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animal welfare is a key consideration in all of our protocols and we will be guided by our NACWO and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. We will continually review our procedures from a welfare standpoint to identify any potential for refinement. We will use the most refined handling techniques.

For example, post-operative care and pain management are critical refinements for the sponge angiogenesis assays . We will seek to refine the administration of the substances to assess vessel perfusion, hypoxia and tumour cell proliferation by evaluating if administration via the more refined intravenous or subcutaneous routes would result in the adequate delivery to the tumour or sponge.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Information from the NC3Rs will be regularly reviewed and the following publications consulted.

PREPARE: guidelines for planning animal research and testing. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.PMID: 28771074).

The LASA guidelines: RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare
and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)

Guidelines for the welfare and use of animals in cancer research Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA; Committee of the National Cancer Research Institute. Br J Cancer. 2010 May 25;102(11):1555-77. doi: 10.1038/sj.bjc.6605642.

Angiogenesis assays – A critical appraisal of current techniques (ed Staton, Lewis and Bicknell) 2006 Wiley.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay updated via literature searches, seminars and conferences to find out about new technology and new approaches that we could implement.

We will comply with the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments; www.nc3rs.org.uk/arrive), a NC3Rs-developed checklist of the essential information that should be included in publications reporting animal research.

We will regularly review guidance from the NC3Rs and implement any changes that would lead to the refinement of our work and sign up to the NC3Rs newsletter..

Home Office

NON-TECHNICAL SUMMARY

191. The role of clotting proteins in inflammation and immunity

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

thrombin, inflammation, atherosclerosis

Animal types	Life stages
Mice	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Most are aware of the role that clotting proteins, such as thrombin, play in blood clotting. But few are aware that they also play a role, independent from clotting, in inflammation and immunity. This project will investigate the cellular and molecular mechanisms by which thrombin and other members of this family of enzymes influence the inflammatory responses involved in three different models of human disease; development of fatty arteries (atherosclerosis), fibrous narrowing of blood vessels (intimal hyperplasia), and an archetypal response to foreign proteins (delayed type hypersensitivity) that underpins many human immune responses. It will also explore the potential to use novel therapeutics to influence these fundamental processes

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There are several reasons why this project is important;

a) Inhibiting clotting proteins has a profound effect on inflammation in many animal models, but translation into the clinic has been hampered by the impact that anti-coagulants have on bleeding, which can be serious, if for instance patients experience bleeding into the brain. We have developed a way, for the first time, to uncouple the effects of an anti-thrombin on clotting from inflammation, so that when used, animals are prone to excessive bleeding for only 1/7th of the time that inflammation is inhibited. Part of this project will be to test this and other novel reagents in different models of inflammation, as part of an exploratory programme to advise on potential clinical uses.

b) If we can work out the molecules, receptors and cells involved in how thrombin and other clotting proteins influence inflammation, we may be able to work out ways to inhibit the thrombin effect without affecting clotting and bleeding, so the other part of this project is to work out how these proteins influence different aspects of the inflammatory and immune response, to discover novel routes of inhibition.

What outputs do you think you will see at the end of this project?

Several primary outputs are envisaged:

a) Publications: we have a good track record of converting our work into primary publications in high quality peer-reviewed journals

b) Therapeutics with clearly defined clinical application. This will be obvious from our published work. We are actively pursuing sources of funding to enable translation into the clinic. For PTL060, this has undergone pre-clinical development resulting in toxicity data and GMP material and its ready for first in man studies. For the newer 'cytotopic' agents which are the prime focus of this project, it is hoped similar development will flow from this new work

Who or what will benefit from these outputs, and how?

Short-term: my team members doing the work will benefit from the experience of designing and performing experiments, reviewing and auditing procedures and data, presenting and publishing their work, and in some cases obtaining a PhD thesis. I will also benefit from their success. 'Short-term' begins before completion of this project

Medium term: the scientific community will benefit from the new knowledge gained, which will hopefully be built upon by other groups to advance the field. 'Medium term' begins before completion of this project and extends into the distance

Longer term: assuming we are successful at showing the potential of our new therapeutics, patients may

benefit from translation of our findings into novel drug treatments. 'Longer term' begins after completion of the project and may be dependent on further projects **How will you look to maximise the outputs of this work?**

Submission of abstracts and manuscripts to meetings and journals (respectively)
Presentation of data at meetings and seminars during local, national and international conferences
Use of our webpages to publicise high impact findings
Timely submission of new grant applications, with appropriate collaborations, to continue down fruitful avenues of exploration
Submission of patents, if appropriately novel findings warrant

Species and numbers of animals expected to be used

- Mice: 2900

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Juvenile and adult mice have been chosen because of the vast array of reagents available, the number of genetically modified strains available to work with and the relative in expense of working with these animals compared to larger species.

Typically, what will be done to an animal used in your project?

Some animals will be maintained for breeding only. In all other protocols, most animals will receive at least one injection of non-toxic substances, and in some protocols, blood will be sampled at various time points. In the fatty artery model (atherosclerosis), the only other fixed intervention is that most mice will be given a high fat diet to eat. In the archetypal immune response model (delayed type hypersensitivity), mice will be exposed to foreign antigen on the skin twice, first on the abdomen, and second on the ear lobe (pinna). In the fibrous narrowing of blood vessels model (intimal hyperplasia), mice will undergo recovery surgery, with a small scar on the front of their upper thorax/lower neck.
Up to 20% of the mice in the non-breeding protocols will receive radiation treatment to facilitate bone marrow transplantation before undergoing the above procedures approximately 1 month later.

What are the expected impacts and/or adverse effects for the animals during your project?

Radiation treatment followed by bone marrow transplantation causes weight loss in all animals, of approximately 8% of body weight, which begins to recover beyond day 5. Most mice receiving the maximum dose of radiation are expected to develop mild self-limiting dry eyes and later, a degree of cataracts, but neither is expected to cause pain, discomfort or distress. The other potential major harms associated with radiation treatment and bone marrow transplantation include severe diarrhoea, breathing difficulties and an inflammatory disease called ‘graft versus host disease’. Only 1% or fewer suffer these serious side effects.

Approximately 20% of animals fed a high fat diet in the fatty artery model develop skin irritation followed by severe scratching (excoriation), which can be managed to relieve pain and distress, if necessary, by claw trimming and antibiotic ointments.
Recovery surgery in the fibrous narrowing of blood vessels model is rarely (<1% animals) followed by signs of a blocked blood vessel causing a 'stroke'. If this occurs, it is obvious immediately post-recovery so animals will be killed straight away to minimise suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

A minority of mice (≤20%) undergoing radiation treatment followed by bone marrow transplantation have been ascribed a moderate severity level. Up to 20% of genetically modified mice on a high fat diet can develop skin irritation and scratching which is also ascribed moderate severity level. The remaining mice are expected to suffer only mild levels of distress or morbidity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Each of the models we are studying involves multiple different elements of the immune system and also elements of repair/healing that respond in a complex and coordinated way to either blood vessel inflammation due to altered fat metabolism or blood vessel injury after a wire insertion, or foreign protein antigens applied to the skin. Each of these is a model of human disease.

Which non-animal alternatives did you consider for use in this project?

We already use non-animal, 'test-tube' alternatives where possible, to minimise animal use. Therefore, we use petri-dish cultured cells to study the impact of clotting proteins on cell growth, differentiation, death and protein production. With regard to the application of protein antigens to the skin, our interest is in the involvement of cells other than T lymphocytes, for which there is no 'test-tube' counterpart.
In addition, we are using laboratory models of human organ perfusion to study the impact of our therapies on blood clotting.

Why were they not suitable?

There are no suitable test-tube (or other models) of fatty artery regression, fibrous narrowing of blood vessels or archetypal non-T cells responses to protein antigens.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This has been done in two ways. First, I have examined the number of animals used on my existing licence for the last four years, which has been particularly useful to estimate the number of future mice to breed, as I envisage no major change in the overall nature of the work I will be performing. Second, i have tried to assess the number of experiments to be performed, and numbers per group, considering all controls, and used this to estimate the mice used in procedures

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I have taken extensive advice, in the past from statistical colleagues. The basic design of in vivo experiments has not altered over the years; all involve comparison of a control group with an experimental group with a specific biological read-out. Use of 6-7 animals per group, in each experiment, will enable the differentiation of a 20% difference in read-out between groups at 90% power and 5% significance. We have already performed pilot dose-comparison studies on a previous PPL to determine the minimum effective IV dose of the cytotopic proteins.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We plan to collect all tissue, blood and cells from every animal used in each experiment, and store appropriately, in a local tissue bank in our laboratory, to avoid having to repeat experiments to examine new aspects of the model - we can instead revert to stored tissue to ask exploratory questions, only having to use new animals if our exploratory findings are positive.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Compared to previous licences, I have limited the number of models to be studied to three; dietary modification to result in accelerated fatty artery inflammation in genetically modified mice, development of ear swelling after protein antigen exposure, and development of fibrous narrowing of blood vessels after mechanical damage. These three represent models that encompass three fundamentally different immune and repair processes, that will allow my group to extrapolate findings to multiple diseases. That the scientific community will embrace this approach is best illustrated by the fact that one of my PhD students won a major award at national meeting of transplantation experts in 2020, despite the fact that her presentation was entirely based on results from the ear swelling model: this is because the type of immune response that develops is representative of that which develops to all localised antigens, including transplanted organs. Despite being a transplant immunologist, this is my first licence that does not contain transplant models.
The fatty artery inflammation and archetypal immune response model are essentially 'mild' in severity, but classed here as moderate because a minority of the animals will undergo radiation treatment followed by bone marrow transplantation to allow us to dissect underlying mechanisms. The majority of animals will suffer no pain or distress. As stated below, we will adopt contemporary methods to minimise distress associated with repeated handling and injections and use local anaesthetic appropriately. In the third model, involving recovery surgery, we will use peri-operative analgesia, and animals will be closely monitored for signs of distress. All experiments will be terminated at the earliest time point that enables best measurement of the endpoints under study.

Why can’t you use animals that are less sentient?

The three disease processes being studied are diseases of mammals. The mouse represents the smallest of the models that are used by investigators in the field (the others being rabbit and pig). The types of immune response to be studied take longer than a few hours to develop, and for practical purposes we need juvenile or adult mice of the appropriate size (for instance with big enough arteries in which to insert a fine wire) to enable efficient and reproducible study.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I will endeavour to stay abreast of advances in all areas. As an example of how we can refine our procedures, several of our models involve repeated handling (for injection, for instance). We will stop using the tail to pick up mice and instead use cupped hands (or a tunnel if available) to minimise distress and optimise reproducibility of our data.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We aim to report our work according to the latest ARRIVE guidelines (2.0), and in doing this will help ensure our experiments, from the planning stage, are performed in the most refined way.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I will use the NC3R website as the primary way to stay informed about the latest advances in the 3Rs

Home Office

NON-TECHNICAL SUMMARY

192.The role of genes in blood vessel formation

Project duration

3 years 0 months

Project purpose

- (a) Basic research **Key**

words

blood vessels, development, Protocadherin 1

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to assess the role of genes in the formation of blood vessels. Blood vessel formation is an essential process in development of organisms and it also plays a pivotal role in a number of diseases including cancer and inflammatory disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to undertake this work because we are not able to model all aspects of blood vessel formation in vitro. This is because there is a complex interaction between a number of different cell types which drives this process. It is known that vessel formation is strongly driven by low levels of oxygen. Then as new vessels form and blood flows, there is increased oxygenation of tissues terminating the signals for this process. Modelling these dynamic changes in oxygen and the element of blood flow is not yet possible in vitro. Understanding how blood vessels form has the potential to yield novel therapeutic interventions for diseases where vessel formation contributes to the pathology of the disease; this included solid tumours, chronic inflammatory diseases such as rheumatoid arthritis and eye diseases including diabetic retinopathy and age related macular degeneration. There are also diseases, including ischaemic heart disease, where a better understanding of vessel formation, may enable the development of therapies that will drive vessel development to restore blood flow to damaged tissue.

What outputs do you think you will see at the end of this project?

At the end of this project we will generate new information about the function of PCDH1 and whether this gene products plays a role vessel formation both during development and in the adult. Information will be disseminated in the form of conference presentations and publications. Indications that PCDH1 does regulate vessel formation could yield novel therapeutic strategies for manipulating vessel formations in diseases such as cancer where this process contributes to the pathology. Thus, positive results could generate further funding to explore these options.

Who or what will benefit from these outputs, and how?

In the short term this project will yield novel information about the role of PCDH1 in vessel development, this has not been investigated and so our findings will be of benefit to the scientific community. They will gain a greater understanding of the function of this gene and gain greater insight into the role of this family of genes as a whole. In the longer term, if we discover a role for PCDH1 in vessel development, this will yield the potential for future funding to investigate ways of modulating PCDH1 function to regulate the process of vessel formation, This could have impacts on diseases such as cancer, since solid tumours depend on vessel formation for their growth and metastasis. In addition, discovering new ways to promote vessel formation could impact upon the treatment of heart attack or peripheral artery disease where promotion of new vessel growth would be beneficial.

How will you look to maximise the outputs of this work?

We will publish our work in open-access peer-reviewed journals in accordance with the ARRIVE guidelines and present at conferences to disseminate new knowledge about the function of this gene. Any results, including negative data, about the extent to which loss of PCDH1 affects vessel formation will be reported. We collaborate with a group external to this university on this gene and our results will be of benefit to them. We will also share our findings with groups internal to the university who have an interest in some of the proteins with which PCDH1 interacts.

Species and numbers of animals expected to be used

- Mice: 200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice as the process of vessel formation has been well studied in this species and closely resembles that found in humans. In addition, the technology of manipulating the genome is well developed in mice enabling us to investigate mice lacking our gene of interest in either all cells or cells of a particular type. The tissue distribution of our gene of interest PCDH1 in mouse very closely resembles that of human PCDH1; this not the case for lower organisms such as zebrafish which can be used to study the function of some genes in vessel formation.
Because we are investigating developmental blood vessel formation we will evaluate the vascularisation of embryos and also in the retina, where vessels develop shortly after birth. To study blood vessel formation in adult mice, we will also use mice at this stage of life.

Typically, what will be done to an animal used in your project?

The project involves breeding mice which lack PCDH1 in either all cells or just endothelial cells, the cells that line blood vessels. We will then examine blood vessel development at various stages of development in mice after they have been humanely killed. Of particular interest will be to examine vessel development in embryos just prior to mid-gestation and the vessels of the retina examined shortly after birth. We will also investigate vessel formation in adult mice in two contexts. In the first we will examine vessels that develop in tumours. To investigate this, tumour cells are implanted under the skin into the flanks of mice. The size of the tumours, which take about 3 weeks to grow, will be monitored using callipers (that provides precise measurements in millimetres). Once the tumours have reached their size limit the experiment will be ended and the mice killed, as well as measuring the rate at which the tumours grow the tumours will be examined for various parameters of vessel formation and functionality. We will also use a second method to investigate vessel formation in adult mice, this is driven by different growth factors more associated with wound healing, in this model small pieces of sponge are implanted under the skin under anaesthetic, these sponges are then injected with factors to promote vessel development every 2-3 days for two weeks. The mice are then humanely killed and the sponges are removed and examined for vessel growth.

What are the expected impacts and/or adverse effects for the animals during your project?

We do not expect the breeding to be associated with adverse impacts, reports from our collaborators suggest that mice lacking PCDH1 are smaller than those that do not lack PCDH1 but are otherwise healthy.
Mice implanted with tumour cells will experience tumour growth which will not cause any pain. When the tumour is become larger, they can impede the normal behaviour of the mouse. Rarely the skin around the tumours may ulcerate. Animals will be killed according to monitoring for adverse effects set out in the protocols.
Mice implanted with sponge will suffer discomfort immediately after the implantation of the sponge, however analgesics are administered to minimise the pain. After this has healed the mice tolerate the implanted sponge well and the injections into the sponge are done while the mice are lightly anaesthetised and this does not result

in adverse effects. Rare adverse effects associated with poor wound closure and infection can occur and if they do animals will be killed according the monitoring for this as set out in the protocol.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The breeding protocol is associated with a moderate severity, this is because mice lacking PCDH1 in all cells are smaller and there is a greater pre-weaning loss of these mice. Though smaller they do not show any signs of ill health. We expect to see this severity in around 25% mice.
The tumour implantation protocol has a moderate severity expected in 90% of the animals, it is probable that in 10% of the animals the tumours may not grow properly.
The sponge implantation protocol has a moderate severity in all animals, this is because the severity is associated with surgical implantation of the sponge which occurs in all animals undergoing this protocol. Once implanted the sponge does not change in size.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We use a number of non-animal systems which replicate limited aspects of vessel formation to investigate genes of interest before considering the use of animals to more fully explore the functions of these genes. Animals are needed to assess blood vessel formation because at the moment there are no laboratory models that can fully replicate this process. This is because it is not yet possible to incorporate the different types of cells, signals and the aspect of blood flow that are all involved in driving vessel development.

Which non-animal alternatives did you consider for use in this project?

We have explored a number of experimental systems that replicate aspects of blood vessel formation. The models that best replicate this process involves co-culturing endothelial cells, which are the cells which form new vessels, with other cell types that can provide supporting signals and scaffold. The Chick embryo CAM chorioallantoic membrane (CAM) model is another method that can be used to investigate blood vessel formation.

Why were they not suitable?

Our co-culture model promotes the formation of endothelial tubes, but these do not fully resemble vessels found in the animal and the aspect of blood flow is missing. It is not yet possible to fully replicate the conditions found in the animal which cause blood vessels to be formed. We are constantly reviewing and exploring new non-animal systems to try to better model vessel development. We are currently investigating the possibility of using stem cells which can be differentiated into endothelial cells and used in organoid systems which support the formation of vessel like structures. While these models show promise they do not yet fully replicate the process of vessel formation as it occurs in animals. While the chick embryo CAM model has been very useful in testing

the angiogenic properties of various factors, it would not be suitable for investigating PCDH1 because we are not able to undertake genomic modifications in the chicken and expression of PCDH1 in chicken has not been reported to have a vascular expression pattern.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers have been estimated based on our previous experience with these experimental models combined with the use of statistical tools (power analyses) to determine the numbers of mice required to give meaningful data. Given that our gene of interest has not been previously studied in the context of vessel formation, effect size will be determined small pilot experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The NC3Rs experimental design tool and its associated statistical analysis tool has been used to assist with experimental design and reduce the number of animals used. We will use methods to reduce subjective bias, and maximise the information obtained from a minimal number of animals by, for example, longitudinal studies monitoring tumours as they grow.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use the most efficient breeding strategies to generate mice of the correct phenoytpe and monitor breeding closely so as not to generate excess animals. All experiments will be designed in line with the PREPARE guidelines checklist to ensure the best possible design. In addition, data will be published in Open Access Journals and in accordance with the ARRIVE guidelines. Negative data, should it be generated will also be reported.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The angiogenesis assay we propose in this application is the rodent subcutaneous sponge model. This model is robust, sensitive and reliable with the result that we use a minimum number of animals for each experiment. This model is the most refined being far less invasive –than other angiogenesis assays typically used such as the rabbit corneal assay, skin flap assays or the dorsal air sac assay. The subcutaneous implantation model of

tumour growth is one of the most refined tumour models combining ease of implantation by subcutaneous injection and facile monitoring of tumour growth.

Why can't you use animals that are less sentient?

Previous research by many groups has documented that the mouse is the lowest organism with sufficiently similar angiogenic patterns to man to allow meaningful evaluation of mechanisms of blood vessel formation. It is possible to use vessel development in zebrafish embryos to determine the role target genes may play in developmental angiogenesis. However, use of this model is contingent on the genes of interest having zebrafish orthologues with similar expression patterns to their mammalian counterparts. The gene of interest in this study has been duplicated in the zebrafish genome and has an altered expression pattern in this species. By contrast, mouse and human show similar expression patterns of the gene being studied in this licence application. We will evaluate vessel development in mouse embryos from day 9.5 – 13, during which time the early vessels and first angiogenic vessels are formed, and before embryos are covered by ASPA.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animal welfare is a key consideration in all of our protocols and we will be guided by our NACWO and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. We will continually review our procedures from a welfare standpoint to identify any potential for refinement. We will use the most refined handling techniques.

For example, post-operative care and pain management are critical refinements for the sponge angiogenesis assays . We will seek to refine the administration of the substances to assess vessel perfusion, hypoxia and tumour cell proliferation by evaluating if administration via the more refined intravenous or subcutaneous routes would result in the adequate delivery to the tumour or sponge.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Information from the NC3Rs will be regularly reviewed and the following publications consulted.
PREPARE: guidelines for planning animal research and testing. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.PMID: 28771074).

The LASA giudlines: RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.)
Guidelines for the welfare and use of animals in cancer research Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA; Committee of the National Cancer Research Institute. Br J Cancer. 2010 May 25;102(11):1555-77. doi: 10.1038/sj.bjc.6605642.
Angiogenesis assays – A critical appraisal of current techniques (ed Staton, Lewis and Bicknell) 2006 Wiley.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay updated via literature searches, seminars and conferences to find out about new technology and new approaches that we could implement.
We will comply with the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments; www.nc3rs.org.uk/arrive), a NC3Rs-developed checklist of the essential information that should be included in publications reporting animal research.
We will regularly review guidance from the NC3Rs and implement any changes that would lead to the refinement of our work and sign up to the NC3Rs newsletter.

Home Office

NON-TECHNICAL SUMMARY

193.The role of neurohormones in the control of brain circuitries and behaviour

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Physiology and behaviour, Mechanisms of regulating brain activity, Neurohormones as signalling molecules, Social behaviours, Neurological disorders

Animal types	Life stages
Rats	neonate, juvenile, adult, pregnant, embryo, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project examines the importance of neurohormones, in particular oxytocin and vasopressin, in the control of brain circuitries and behaviours.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Within the brain, the neurohormones vasopressin and oxytocin are synthesised by a number of different populations of nerve cells and these different populations are responsible for specific neurohormone-dependent behaviours. Understanding how vasopressin and oxytocin trigger behavioural effects; including appetite, sexual and aggressive behaviours, social interaction, maternal care and bonding has attracted widespread attention because of the possibility that drugs mimicking peptide actions may be valuable therapeutically. For vasopressin and oxytocin, there is evidence of links with neuropsychiatric disorders, including eating disorders, stress disorder, anxiety, depression and autism.

What outputs do you think you will see at the end of this project?

This project will address fundamental principles in neuroscience and is therefore likely to have implications for many neurological disorders such as autism and obesity. As such, the primary output will be generation of knowledge that will be largely disseminated by publication in peer reviewed journals and presentation at conferences, seminars and workshops. I will also continue active participation in public engagement events, media interviews and our institute’s Public Engagement and Communications Committee to increase the public’s awareness of our work and its implications for health.

Who or what will benefit from these outputs, and how?

The ‘discovery research’ nature of this project means that the short-term beneficiaries will primarily be the academic community. Our previous work has led to high-impact publications and this has given us a high profile in the academic community.

The outcomes of this project could also steer future research towards new therapeutic strategies using neuropeptides and hence will be of long-term benefit to the pharmaceutical industry and charitable organisations that fund medical research and ultimately to human health. The timeframe for improvements to human health would be expected in the decades, while increased investment in Research and Development in these areas could occur within 3-5 years.

How will you look to maximise the outputs of this work?

Findings from this project will primarily be communicated and disseminated through publication in widely-read international peer-reviewed journals, but also presentation at local, national and international congresses and individual institute seminars. To ensure maximum dissemination, only journals with green or gold open access options will be considered.

To disseminate knowledge to the public, we will accompany published papers with targeted press releases, using the University Press Office, and also will target science journalists and use social media channels. It is important that the outcomes of this project are effectively disseminated not only to scientific peers, but also to the general public to help explain how public investment in basic science brings benefits to health and well-being. We have been extremely active in outreach activities, including public lectures, media appearances, science festivals, articles in the popular press, and online briefings and education.

The project has potentially important translational implications, and these will be managed with the support of the University, which has extensive expertise in the protection of intellectual property arising from grant-funded research, and in fostering translational outcomes.

Species and numbers of animals expected to be used

- Rats: 4500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rats will be used throughout this programme of research. Rats have been extensively used as a model organism in this line of research, in our lab and by many others. In term of brain regions and peptides involved in regulating behaviours, the rat is well understood and is comparable to the relevant systems in humans. Using rats lets us avoid repetition of earlier work, builds on current knowledge, and allows direct comparisons of our studies with others.

Typically, what will be done to an animal used in your project?

Some rats will be used for breeding purposes only. Some of the experiments proposed in this study require surgery, for delivery of drugs to specific brain regions and measurements of peptide release. For these experiments, pain will be controlled during surgery by general anaesthesia and pre- and post-surgery by analgesics. The highest severity rating of this programme of work will be moderate but the majority of experiments will have a mild rating. A significant proportion of our experiments will be done under anaesthesia and take no more than a few hours (max 6 hours for long single cell recordings)

What are the expected impacts and/or adverse effects for the animals during your project?

Expected impacts will include appropriately managed post-operative pain in the case of recovery procedures. Transient stress in the case of some behavioural experiments, short lasting sickness in the case of osmotic studies and some weight loss in the case of some dietary restricting experiments. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by rigorous training, correct dosing of anaesthetics, by accurate weighing and maintenance of body temperature during and post-surgery. Risk of infection will be minimised by aseptic techniques. At the end of each protocol, animals will be killed by using approved humane methods and tissues from these animals may be used for post hoc histology. All rats will be monitored closely by experienced staff during the protocols and humanely killed at the end of each experiment, or in the unlikely situation that they present with clinical signs of illness.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately 40% of animals will be used for establishment and maintenance in genetically altered animal breeding programmes or for production of genetically altered rats. These animals will fall will into a subthreshold limit of severity. Of the remainder, approximately half of animals will experience degrees of suffering that fall within a mild severity category, the other half into the moderate category.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this programme of research is to understand how individual and networks of neurons in the brain produce behaviour. To truly understand how the brain processes information, it is imperative that experiments are done at the level of the whole animal. For this reason, it is impossible to avoid the use of animals when addressing the aims of the outlined proposal. However, large amounts of work will be done on in vitro preparations.

Which non-animal alternatives did you consider for use in this project?

There is on-going work in the laboratory using computer models of single neurons. Neuronal models will be used to generate predictions and testable hypotheses based on existing biological data. The use of simple analytical approaches and complex computer models provides a useful method for exploring possible outcomes that can then be tested in rats.

Why were they not suitable?

These approaches are unlikely to replace the need for suitable animal models in which to generate new physiological data. They can however be used to predict hypotheses and design experiments.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals has been estimated based on experience gained under my previous Home Office license. In this regard, the work plan and nature of experiments covered by this project are similar in design to those covered under my previous license. Hence, I have based estimates on average yearly animal use per group member returned under my current license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

For all of our experiments in-bred rats are used to reduce inter-individual differences. We also generally use a randomized block design for experiments to further reduce factors that could cause differences between animals that are unrelated to factors being tested in experiments (e.g. cage to cage variation). By reducing natural differences between animals in this way, we reduce the number of animals needed to identify differences caused our experimental test. In cases where we cannot use randomized blocking to reduce cage effects, we will still ensure that animals are littermate-controlled to reduce variations that can arise between litters. Our experiments are also designed to reduce the number of variables to as few as possible and thereby reduce the number of control groups required. In this respect, we will always consider carefully whether it is important to include ‘naïve’ as well as ‘vehicle’ control groups in experiments, or if the latter alone is sufficient for interpretation of results.

To ensure best practice in statistical analysis and experimental design all new staff members working under this license will attend the in-house ‘Experimental Design Course’. Planned experiments are discussed regularly within group meetings to ensure all are correctly controlled and to facilitate sharing of tissues/data for the most effective use of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

My group routinely perform pilot experiments to determine sample sizes required by power calculations. We standardly base power calculations on the ability to detect at least a 2-fold difference between groups, which we regard as an acceptable cut-off for identifying important biological effects with the benefit of reducing the group sizes required. Experiments are then performed on a minimum of

two separate occasions to ensure reproducibility, following which meta-analysis of data from pooled experiments is used to reveal less pronounced effects but without increasing overall animal use.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats will be used. They are the lowest mammalian group in which neuroendocrine systems are well characterised. We will build on our basis of knowledge and experience in the field, using appropriate and sophisticated techniques to study neuroendocrine neurones in these species. Previous data on which this application is based were also obtained from this species. Experience and the wealth of published literature in these animals has informed and will continue to inform the best use of techniques and choices of experimental approaches that are optimal for minimising potential adverse effects.

Why can’t you use animals that are less sentient?

Adult rats will be used. They are the lowest mammalian group in which neuroendocrine systems are well characterised. Recovery surgical procedures, behavioural testing and conscious animal experiments cannot always be replaced by alternatives.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The department that manages the University’s animal facilities includes a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. My group will consult closely with them and take full advantage of the extensive resources provided by them to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures. The University is also in the process of adopting the improved rodent handling methods that reduce animal stress (detailed by Hurst et al. Nat Methods 2010) and now provide environment enrichment as standard. Singly housed rats will be housed in clear open topped cages to allow visual and olfactory contact with their neighbours. In experiments that require handling of the conscious rats the animals will be habituated to the experimenter in order to reduce stress. My group will adopt these methods alongside the animal facility staff. Anaesthesia and analgesia will be provided where suitable. To reduce infection risk, the best aseptic technique will be used during surgery (e.g. sterilization of instruments between animals, full surgical drapes). We will also routinely consult the NC3R’s website (nc3rs.org.uk) and take full advantage of the annual 3R’s seminar day organized by the University’s Animal Welfare Committee to find out about pioneering developments in best practice and methods to improve animal welfare.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Our institute employs a dedicated team of veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consults closely with this team and takes full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures. We have also consulted the NC3Rs research strategy paper by Prescott MJ, Lidster K (2017). We also regularly review the scientific literature in the field to keep abreast of current best practice in experimental design and approaches.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our institute employs a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consult closely with this team and take full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures that are continually being updated. Our university is also in the process of adopting the improved rodent handling methods that reduce animal stress (detailed by Hurst et al. Nat Methods 2010) and our animal facilities now provide environment enrichment as standard. My group will adopt these methods alongside the staff in our animal facilities. We will also take full advantage of the annual 3R’s seminar day organized by the University’s Animal Welfare Committee to find out about pioneering developments in best practice.

Home Office

NON-TECHNICAL SUMMARY

195. Therapeutic targeting of C-reactive protein

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

C-reactive protein, drug discovery, inflammation, tissue damage

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Development of new drugs to target the adverse, tissue damaging effects of C reactive protein (CRP).

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our laboratory was the first to show that human CRP exacerbates tissue damage after ischaemic necrosis in heart attacks and strokes and to validate CRP as a therapeutic target in these diseases. We have also highlighted the role of CRP in pathogenesis of a wide variety of other tissue damaging conditions, including trauma, infection, immunoinflammatory diseases and cancer cachexia. CRP contributes to disease severity in influenza and other human respiratory virus infections, including the current Covid-19 pandemic. We have invented a family of CRP inhibitor compounds, the drug candidate from which is now in accelerated development towards first in human testing and the earliest possible clinical efficacy studies in patients with Covid-19. The work for which the present licence is sought is critical for uninterrupted development of this potential medicine, which should reduce morbidity and mortality from Covid-19.

What outputs do you think you will see at the end of this project?

We will have identified one or more CRP inhibitor compounds suitable for investment of the tens or hundreds of millions of pounds required to develop them into licensed medicines for use in patients to treat unmet medical needs. After ensuring the robust patent protection that is essential for creation of any new medicine, we will certainly report our findings in high impact peer reviewed scientific and medical journals.

Who or what will benefit from these outputs, and how?

We confidently expect that within the duration of this licence, one or more CRP inhibitor compounds will have progressed through the development process to become licensed medicine(s) being used for patient benefit. As previously noted in this application, the diseases for which there will be potential benefit include infections, inflammatory diseases, trauma and cancer.

The obvious proviso is that there is never a guarantee of drug development success. Candidates can fail at any stage in the process, either through adverse effects or lack of efficacy. Although the supportive evidence is overwhelmingly strong, the only robust test of our underlying hypothesis about the pathogenicity of human CRP will be the demonstration of efficacy of CRP inhibition in human clinical practice. Progression of drug development to enable such a demonstration depends on the work for which the present licence is sought.

How will you look to maximise the outputs of this work?

We are already supported by a consortium of UK universities and major international pharma companies, whose purpose is to develop new medicines from the inventions and discoveries made in its constituent universities. They will energetically ensure that an effective compound from our work is licensed to the optimal pharma company for progression through to medicine licensing and commercial production. In addition, we are in direct discussions with a number of suitable major international pharma companies, in order to have alternative commercialisation opportunities. As soon as patent applications have been filed on our new inventions, the work will be as widely reported as possible in scientific and medical media, as well as in clinical educational literature of all types, so that clinicians are promptly made aware of this novel, and we hope significant, new therapeutic intervention.

Species and numbers of animals expected to be used

- Mice: 350

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult mice are ideal for the present project because well developed and thoroughly characterised models exist for all of the pathological process and diseases to be studied, closely resembling their human counterparts.
Typically, what will be done to an animal used in your project?

Most animals used in this project will be injected with substances and/or receive subcutaneous osmotic pump implants. Most inflammation experiments will be completed in 2-3 days and none will last longer than 7 days. Most PK/PD studies will also last only 2-3 days and none will exceed 28 days.
What are the expected impacts and/or adverse effects for the animals during your project?

There will be some local soreness/irritation of the skin around the wound following the osmotic pumps implantation, with discomfort for 2-7 days post operatively. Dehiscence, weight loss and post-surgical infection, can occur, but are uncommon (<5% of animals).

The Shwartzman reaction, which occurs in response to LPS injection, is expected to be accompanied by discomfort, which may be recognised by scratching or licking of the injection site. The 24 hour interval after LPS injection is required to allow consistent development of the Shwartzman reaction, while minimising the duration.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For the whole project we expect that about 70% of the animals will be in the mild severity category and about 30% in the moderate category.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The initiation, development and evolution of pathology causing clinical diseases in living organisms are extremely complex. Similarly the mode of action and efficacy of disease modifying therapeutic interventions are also very complex, as are the PK and PD of all therapeutic agents, including those to be studied here. There is

no alternative to use of living animals to achieve the level of knowledge and understanding of these processes which is necessary for the new approaches to treatment which we have invented to be taken into clinical testing in humans.

Which non-animal alternatives did you consider for use in this project?

No existing or conceivable in vitro studies can possibly provide the necessary information and in vivo animal testing will in any case be a legal and regulatory requirement for drug development. Only candidates tested in vitro and bound with greatest affinity by the human CRP target will be tested in vivo.

Why were they not suitable?

See above.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We will use only the standard experimental protocols with which we have extensive experience that we have established to provide robust, statistically rigorous results. We expect to undertake approximately 10-15 studies using 20-30 mice in each

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Our experimental design replicates precisely the protocols we have been using for these and similar studies over the past 50 years under the Project Licences held by my predecessor and then myself.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We decided to use wild-type C57BL/6 mice in this project in order to keep the number of animals as low as possible. We won't have to breed a colony of GA animals and only use some of them as we did in previous studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are ideal for the present project because well developed and thoroughly characterised models exist for all of the pathological process and diseases to be studied, closely resembling their human counterparts. In the case of the Shwartzman reaction, some dermal inflammation is necessary to obtain the desired result of

demonstration its reduction. Our model, with killing of the mice as soon as possible, involves the minimal possible pathology consistent with this requirement.

Why can't you use animals that are less sentient?

Humans are warm blooded (endothermic) and are very different from cold blooded (exothermic) animals. All aspects of human organ, systems and whole body physiology and pathophysiology are markedly different from those of fish, amphibians and non-protected species. Thus, although proteins homologous to human CRP exist in many species, the acute phase response of plasma proteins, which is a key feature of the endothermic animal response to injury, infection and inflammation and which underlies the pathogenicity of human CRP, does not exist in exothermic animals. In contrast, many relevant aspects of murine and human physiology and pathophysiology, specifically including those related to C-reactive protein, are very similar. There is also a substantial body of relevant knowledge in the mouse, to which we have previously made the most substantial contributions. The mouse is therefore the only species suitable for our required studies.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

If we can elicit a less intense inflammatory response in the Shwartzman reaction and still get the output we need to generate sufficient data, we will try to do so. Monitoring, post-operative care and pain management will always be part of our priorities.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will always try to follow the guidelines issued by LASA and NC3Rs when possible.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

NC3Rs resources (website, publications...).

Home Office

NON-TECHNICAL SUMMARY

196. Therapies to Protect and Regenerate the Newborn Brain following Perinatal Injury

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

neonatal encephalopathy, neuroprotection, therapeutic hypothermia, birth asphyxia, inflammation sensitisation

Animal types	Life stages
Pigs	neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this project is to reduce death and disability in newborn babies who have complications around the time of birth. These complications include lack of oxygen, infection/inflammation or a combination of the two and lead to an abnormal conscious state and problems with breathing called **neonatal encephalopathy (NE).**
Therapeutic hypothermia (HT) or cooling is a partially effective treatment but despite its widespread introduction in high income settings, disability rates remain unacceptably high. However, HT is not routinely given in all settings in low and middle income countries.
The project aims to study therapies given after birth that can reduce brain injury in NE and stimulate regeneration and repair with and without cooling.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

NE is an important cause of death and disability, affecting 1-3/1000 births in the UK and 10-25/1000 births in Sub-Saharan Africa. With current practice of therapeutic hypothermia (HT), mortality of NE has reduced from 25% to 9% and disability from 20% to around 16% with a reduction in the rate of cerebral palsy. However, not all children benefit from treatment and intellectual impairment may remain even in the absence of cerebral palsy. The events which follow a period of lack of oxygen around the time of birth are complex and involve changes in blood flow, tissue oxygen levels, abnormal brain transmitters, different types of cell death and inflammation. Optimizing neuroprotection of the new-born requires the use of living models to take into account the influence of other organs, circulating factors and changes in brain perfusion. We are not aware of any alternative that does not use animals that would allow us to achieve this aim.

What outputs do you think you will see at the end of this project?

These studies will provide advancement in the understanding of the beneficial effects of different therapies for babies following a complicated birth with lack of oxygen.
The outputs will be captured on the MRC Research fish site.

- We will seek to engage the public by working closely with relevant Media Relations to publicise our research findings via open-access journals, media interviews, press conferences and releases. As is currently happening with HT, talks would be given at meetings organised by charities such as Bliss, Wellbeing of Women (who supported the original pre-clinical study of melatonin-augmented cooling) and Action Medical Research, at which parent representatives attend. We work with parents of babies with neonatal encephalopathy, and with the Neonatologists treating those babies, in raising the awareness and acceptability of new therapies for brain protection and of clinical neuroprotection trials.

- Professional presentation and publication in high impact journals will enhance the impact of our research and patient benefit on national and international stages. We will use open access publishing for key outcomes to ensure wide dissemination of results.

Who or what will benefit from these outputs, and how?

The information will benefit all disciplines of medicine, including

1. Neonatologists focused on neuroprotection of the term newborn infant in high and low income settings

2. Neonatologists focused on neuroprotection of the preterm infant in high and low income settings 3.

Obstetricians managing fetal distress

4. Paediatricians focused on neuroprotection, for example paediatric intensive care specialists treating HI-reperfusion injuries (e.g. traumatic brain injury and cardiac surgery) in high and low income settings
5. Adult medical specialities treating HI-reperfusion injuries e.g. stroke, myocardial infarction, trauma. HT is already used in out of hospital cardiac arrest and these data will have relevance for single or adjunct therapy
6. Clinicians wishing to conduct clinical trials in babies using outcome bioamarkers (MRS and blood biomarkers)

7. Army doctors and those wishing to protect the brain after injury in the field.

The economy and society will benefit from these studies. NE is the 4th leading cause of death in children and accounts for 50 million disability life adjusted years worldwide. These studies have the potential to reduce suffering from life-long neurologic disabilities and to significantly reduce the societal costs of caring for survivors with neonatal brain injury. The lifetime costs of caring for an individual with cerebral palsy (CP) is estimated at $1.15 million. Using a conservative estimate of 20% CP rates in NE infants treated with therapeutic hypothermia (HT) and conservative estimate of NE incidence of 2/1000 in high resource settings, the economic burden in the US would be $1.7 billion in lifetime costs (similar pro-rata costs in the UK). NE is also estimated to produces additional lifetime costs of $1.6 billion for intellectual disability from NE. HT has been shown to reduce the burden of these costs. If these therapies augment cooling, these costs will be reduced further.

How will you look to maximise the outputs of this work?

Dissemination of New Knowledge

Confirmation of the safety and efficacy therapies with and without hypothermia, in males and females will provide a rich source of information for future clinical trials in babies at risk of brain injury after a complicated birth. We will set up a Brain Protection website as a focus for parents looking for information and support on NE, including treatments. We will increase traffic through this route by marketing the site in social media settings and leading parents in need of support either through the Bliss Parent-line or to seek local professional help. We use the Science Media Centre as a professional support environment for developing media friendly messages and act as expert opinion where required. Press releases for important papers helps raise both public and professional profile of the study. We will work with a range of national and local charitable organizations via lectures, hospital visits and event support. We plan to hold two meetings throughout the project (evening meetings with a councillor and refreshments) to showcase the potential benefit of therapies, whilst maintaining discretion about the translational nature of our work. Feedback and opinion on the acceptability of our studies from parents are vital to the success of future clinical trials.

Collaboration

Our findings could change the trajectory of perinatal neuroprotection both in the UK and worldwide. Our team works closely with an international cohort of Neonatologists and Perinatal Neuroscientists in developing standards of care for perinatal neuroprotection. This work will inform an urgent review of those standards, including an update to the UK NICE Cooling guidance (last updated 2010).

Species and numbers of animals expected to be used

- Pigs: 1250 piglets

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The neonatal piglet has significant strengths compared to the rodent for neonatal neuroprotection research: the size allows for the same intravenous lines and catheters to be used as in babies, the medications are based on NICU protocols and the maturational stage with a brain growth spurt around the time of birth is similar to human brain.

Typically, what will be done to an animal used in your project?

The animal is sedated and transported to the designated facility. The animal is given an inhaled anesthetic and remains insentient throughout the study.

1. A breathing tube is inserted down the windpipe and the animal's breathing performed by the neonatal ventilator throughout the study.
2. Catheters with remotely operated balloons are gently placed round the carotid arteries that supply the brain.
3. Central lines (for infusing fluids and sampling blood non-invasively) are inserted through the umbilical cord.
4. A short period of reduced oxygen and blood supply is induced after surgery and stabilization
5. An injection of lipopolysacchride may be given before the period of lack of oxygen in some piglets
6. The studies will typically last 72h (in some cases where we are assessing seizures, we will perform shorter studies of 12-36h)

What are the expected impacts and/or adverse effects for the animals during your project?

Expected adverse effects and likely incidence

General anaesthesia will be maintained and hence the animal will be insentient throughout the entire procedure. If any unforeseen complications with anaesthesia or monitoring occur that cannot be rectified the animal will be terminated.

How the adverse effect will be recognised

Complications with the anaesthesia will be recognised by changes in the heart rate and movement of the piglet.

Refinement control measures

We have an isoflurane meter installed so that we can see immediately the isoflurane concentration delivered to the piglet and can rectify any problem with isoflurance delivery immediately. Humane end-

points and limits of severity

With any unforeseen complications that cannot be rectified, the animal will be terminated.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The severity classification is AC - unclassified code. All studies are terminal.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Optimising neuroprotection of the newborn requires the use of in vivo rather than in vitro models to take into account the influence of other organs, circulating factors and changes in cerebral perfusion.

Which non-animal alternatives did you consider for use in this project?

We are not aware of any alternative that does not use animals that would allow us to assess the safety and efficacy of therapies for birth asphyxia. A model is needed in which meticulous intensive care and temperature control can be maintained.

Why were they not suitable?

Mathematical modelling could not replace the use of experimental animals. The body of knowledge about therapies and combinations of drugs is not sufficient to allow for a clinical trial in human infants without these pre-clinical studies in the piglet providing important, safety, dosing and efficacy data.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We use the minimal number of animals in each group to be able to show a biologically important neuroprotective effect in our pre-defined primary outcome measures. The group size is based on the data variability in previous studies. The proposed experimental designs and methods of analysis have been discussed with our statistics

department and other biomedical statisticians. The outcome measures (aEEG and brain lactate/NAA biomarkers and immunohistochemistry) are quantitative and random effects regression is an appropriate statistical analysis for this. To reduce the effect of the insult severity and thus reduce group size, we include the insult severity index in the analysis.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We are aware of the NC3R's Experimental Design Assistant and work within this framework. Studies adhere to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.
The potential sources of bias in the study (for example time of day or season of the year) have been addressed Developing the model further will include both male and female animals. Cell death pathways differ between sexes. We will use the minimal number of animals to ensure we can detect a sex difference in response to therapies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We use pilot studies to ensure optimal dose and therapeutic levels are known before starting the randomized studies of safety and efficacy. This ensures that wastage and negative studies are avoided due to sub therapeutic drug levels.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use the neonatal piglet with transient hypoxia ischemia and various neuroprotective interventions in this project. The piglet is insentient throughout the study.

Why can’t you use animals that are less sentient?

While a rodent model is typically the first step in testing a
potential neuroprotective treatment, confirmation in a larger animal model provides critical complementary safety and efficacy data to justify applying for the regulatory approvals necessary for clinical trials. The similarity of anatomy, size and maturation of the piglet brain to the human infant allows for regional histopathology and vulnerability of the brain to be assessed. The model allows for meticulous neonatal intensive care support for up to 72h, enabling metabolic and temperature homeostasis to be maintained during this time. This ensures that the model provides data that is relevant to the human neonate who would be cared for with similar intensive care support. The model allows for brain magnetic resonance imaging and spectroscopy studies to be acquired at 30 and 66h; these data are similar to those acquired in babies and therefore adds to the model’s clinical translational relevance.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will continue to monitor the piglets closely throughout the studies, ensuring adequate sedation and anesthesia at all times. The new laboratory will allow for more collaboration with vets and this will refine the model further. Complications with the anaesthesia will be recognised by changes in the heart rate and movement of the piglet. We have an isoflurane meter installed so that we can monitor the isoflurane concentration delivered to the piglet and can rectify any problem with isoflurance delivery immediately.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will adhere closely to the ARRIVE guidelines (www.ARRIVEguidelines.com) from the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will circulate the ARRIVE (https://arriveguidelines.org) and PREPARE guidelines (https://pubmed.ncbi.nlm.nih.gov/28771074/) to the research team and ensure the team are aware and can talk through these guidelines. We will include the NC3Rs as an item on the Agenda at each monthly lab meeting. As we start up a new laboratory, the closer collaboration with vets will refine the model.

Home Office

NON-TECHNICAL SUMMARY

197. Tissue-level control of stem cell identity

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Embryo, Stem Cells, Tissues, Regeneration, Pregnancy loss

Animal types	Life stages
Mice	adult, embryo, pregnant, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how the 3D organisation of a tissue affects stem cell identity and fate during embryonic development and tissue regeneration.

Potential benefits likely to derive from the project, for example how science might be advanced or how

humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

During the development of an embryo and the maintenance of healthy tissues, stem cells are subjected to a number of external factors that can change their identity and fate. An important aspect of how stem cells decide their fate is the immediate environment in the tissue in which they reside, in other words, their spatial three-dimensional neighbourhood. We still know very little about how this process happens. Understanding how stem cells respond to these external spatial cues and decide which cell type to become is important for embryo development and tissue regeneration. During the formation and growth of an embryo, dramatic changes in the shape of the embryo are tightly coordinated with the specialisation of stem cells to form different mature cell types. Alterations in this process lead to pregnancy loss, and therefore studying how embryo shape affects stem cell fate will allow us to explore the potential reasons behind failed embryo development, and potentially to devise strategies to correct it. Similarly, during adult regeneration, stem cells play a critical role to replace damaged tissues.
Studying how external spatial cues affect adult stem cell fate would allow us to devise new protocols for stem cell differentiation, and potentially to develop new strategies to promote tissue repair.

What outputs do you think you will see at the end of this project?

- We will publish our findings in high impact journals

- We will determine how the organisation of embryonic stem cells into an epithelial tissue influences their ability to form the cell types of the mature organism

- We will describe how the formation of specialised regions within a cell determines their fate during development.

- We will analyse whether the organisation of a tissue influences the plasticity of adult stem cells, in other words, their ability to become a different cell type.

Who or what will benefit from these outputs, and how?

The proposed research would contribute to academic progress within and beyond the relevant disciplines:
- Throughout the project: embryonic stem cells are mainly studied in 2D cultures that do not mimic the organisation of tissues in the embryo. The proposed research will clarify how the 3D organisation of the embryo changes stem cell fate decisions. Therefore, the findings will benefit stem cell and developmental biologists. Similarly, the mechanisms that change the identity (reprogram) of an adult cell to an embryonic state have been mainly studied in 2D cultures. The use of 3D *in vitro* models of an organ to study reprogramming represents a novel approach of relevance for the stem cell field.

- Long-term: the proposed research has the potential to impact other disciplines such as human reproduction, regenerative medicine, and cancer biology.

Human embryos are prone to failure during the first weeks of development, but why this happens remains unknown. Understanding how cells commit to a specific fate during physiological conditions is the first requirement to characterise what goes wrong in situations of pregnancy loss.
In terms of regenerative medicine, the knowledge generated during the course of this project has the potential to help us design better protocols and strategies for the *in vitro* generation of tissues for regeneration. *In vivo* reprogramming has been recently proposed as a novel tool to improve aging and induce regeneration. However,

the same strategy may lead to tumour formation. Therefore, understanding how tissue shape affects stem cells may have a direct impact on the development of novel regenerative approaches.
One of the hallmarks of tumour formation is loss of tissue shape. Therefore, understanding how tissue shape changes cell identity will be potentially relevant for the study of cell fate in the context of cancer. **How will you look to maximise the outputs of this work?**

- Generation of tools: genetically modified stem cells generated during the course of this project will be made freely available to academic researchers.

- Dissemination of new knowledge: we will present the results of this project in relevant local, national and international meetings devoted to stem cell biology, organoids, and embryo development. The findings of the research will also be published in high impact journals in accordance with open access policies. Sequencing data will be deposited in appropriate public data repositories.

- Public engagement: we plan to present at events such as the Stem Cell Club, Pint of Science, and Science Week. With the help of a team dedicated to news and public engagement, we will promote our research through the use of social media and news processes.

Species and numbers of animals expected to be used

- Mice: 4,100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse is an ideal model system to study pluripotency - the capability to generate all the cell types in the organism - and embryo development. There are a number of critical tools that are well established in the mouse and allow us to study gene function in a physiological context (e.g. generation of mouse embryonic stem cells - mouse embryo chimeras). Moreover, there is a mouse model already available that allow us to explore the consequences of reverting adult cells back to an embryonic pluripotent state. Since we are interested in pluripotent stem cells during development and regeneration, we will use embryos, pregnant females and adult mice.

Typically, what will be done to an animal used in your project?

Approximately half of the mice used in this project will not undergo any intervention. They will be used for breeding or collection of tissues for *in vitro* experiments.
Approximately half of the mice will undergo an experimental procedure. In most of the cases this will be an injection. We will inject hormones to increase the number of embryos that can be collected from a single female,

and we will inject stem cells to test their ability to form embryonic tumours called teratomas.
To study the development of embryos beyond implantation we will transfer embryos to pseudo-pregnant female mice. This will involve surgical procedures - vasectomy of males and embryo transfers to females. Mice are expected to make a full recovery.
Lastly, we will treat mice with specific compounds, administered orally or via injection, to trigger the expression of specific genes of interest (e.g. pluripotency genes). Expression of these genes will lead to the formation of embryonic tumours.

What are the expected impacts and/or adverse effects for the animals during your project?

Half of the mice used in this project will not experience any pain, discomfort or lasting harm.
Female mice used for superovulation will only experience mild discomfort and will recover fully from the procedure.
Females used for surgical embryo transfers and vasectomised males will experience moderate postoperative pain and discomfort. They are expected to make a full and uneventful recovery.
Mice injected with pluripotent stem cells will develop teratomas in 95-100% of cases. This typically takes around 6-8 weeks. Similarly, mice in which pluripotent genes are induced will develop embryonic tumours in approximately 80% of cases. The formation of embryonic tumours and weight loss will be carefully monitored. Weight loss represents a good indication of the health status of a mouse, and therefore mice will not be allowed to lose more than 10% of their body weight (mild severity). Mice will be sacrificed before tumours are bigger than 1.2 cm, if there are any signs of anaemia, or if there is any deviation from normal behaviour.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Half of the mice used in this project will not experience any pain, discomfort or lasting harm.
Females used for superovulation will only experience mild discomfort following the injection.
Females used for surgical embryo transfers and vasectomised males will experience moderate postoperative pain.
80-95% of mice injected with stem cells and mice in which pluripotent factors are expressed will develop teratomas (mild severity).

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The objective of this project is to understand how the 3D organisation of a tissue regulates stem cell identity and fate during embryonic development and tissue regeneration. The mouse is the ideal model system to achieve this goal because:
- Early developmental processes are similar in mouse and human embryos.

- Mice have short breeding times, consistent with the realistic timeframe of the experiments.

- There are excellent tools available to study molecular mechanisms and manipulate gene expression.

Having said this, we will use alternative *in vitro* models in most of our experiments. Stem cells can be cultured *in vitro* using 3D techniques to mimic the structure of tissues, organs and embryos. These are ideal model systems to discover molecular mechanisms. We will use mice to obtain primary tissues for our *in vitro* experiments, and to validate our *in vitro* findings in a physiological *in vivo* setting.

Which non-animal alternatives did you consider for use in this project?

We will complement our studies using human organoids and human embryos.

Why were they not suitable?

We cannot rely solely on the use of human embryos (to study development) and human organoids (to study regeneration) to achieve our goal.
Human embryos are difficult to obtain and there are numerous technical and ethical limitations to perform genetic manipulations.
The availability of genetically modified mice allows us to readily obtain genetically modified organoids. This is more challenging when working with human organoids, as the genetic modifications need to be introduced de novo.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

- Protocol 1: the number of females needed has been estimated based on our previous experience. We have taken into consideration that: 80% of females treated with hormones (to increase the number of eggs they produce) and mated with a male will become pregnant; from a pregnant female we expect to have an average of 25 embryos; 95% of the recovered embryos will successfully develop *in vitro*. This means that from 2,000 females, 1,600 will be pregnant, and therefore we will obtain 38,000 well developed embryos.

These embryos will be manipulated *in vitro* to generate either chimeras (embryos in which stem cells are injected to study their behaviour) or enlarged embryos (embryos with an increased number of cells), and transferred to females to allow embryo implantation into the uterus and subsequent development. A typical embryo transfer experiment with two groups (control, experimental and rescue) requires approximately 90 embryos (15 embryos per recipient, two recipients per group). Taking into consideration each experiment will be repeated 4 times and 10% of the embryos may not be chimeric we estimate that we will need a total of 20,000 embryos which could potentially allow us to analyse the phenotype of 50 genetic modifications. For size regulation experiments we estimate that we will need 18,000 embryos. This will allow us to generate 6,000 single and enlarged (double-sized) embryos and to explore the function of approximately 20 genes (four conditions -control single, control double, mutant single and mutant double-).
- Protocols 3 and 4: based on the above-mentioned numbers we will need approximately 700 recipients and 50 vasectomised males for the embryo transfers.

- Protocol 5: approximately half of the mice under this protocol will be used to generate new genetically modified animals (by either creating new mutations or by combining multiple alleles to generate a new line), and half will be used in protocols 1 and 7.

- Protocol 6: we plan to use 50 immunocompromised mice to assess formation of embryonic tumours. This will allow us to test the pluripotent status of 10 different stem cell lines.

- Protocol 7: we estimate we will use 300 mice based on previous literature results. The exact number of mice per condition and group will be estimated using POWER statistics based on the proposed *in vitro* experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have taken into consideration the different variables that affect embryo yield. We have estimated the number of mice needed in each protocol based on our previous experience working with embryos and published reports. We will use our proposed *in vitro* experiments to perform POWER calculations and predefine sample size in the different experimental groups before performing the experiments. We have sufficient in-house statistical knowledge to be able to plan the experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

For conventional breedings we will regularly monitor breeding efficiency. We will cryopreserve our strains to maintain only the colonies that are needed at the different stages of the project, rather than perpetuating the strain throughout the course of the project. We will follow the Home Office guidelines for efficient breeding of animals.

To study the consequences of specific genetic modifications for embryo development we will follow a chimera approach (stem cells are injected into embryos to study their behaviour). This will allow us to analyse the genetically modified chimeras at the desired embryonic stage, without generating genetically modified animals. Our experimental approach is based on the use of the 3D *in vitro* models as a tool to screen for phenotypes of interest, and only using the mouse as an *in vivo* model for physiological validation. This greatly decreases the number of animals used for research.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mice as our model system to understand how tissue shape controls stem cell fate. Half of the mice in this project will be used to obtain primary tissue for *in vitro* culture or to generate genetically modified animals, and they will not experience pain, suffering, distress or lasting harm.

When adult cells revert back to an embryonic stage, they give rise to embryonic tumours. We will minimise the suffering caused by the formation of embryonic tumours by restricting our analyses to the initial phases of tumour formation.
Embryonic tumours will be induced by injecting pluripotent stem cells subcutaneously. This is an alternative to the injection of stem cells into the kidney capsule, which involves a surgical procedure. We will therefore avoid surgical procedures.

Why can’t you use animals that are less sentient?

The majority of the animals used for our experiments will be at embryonic stages. This means that the specific mutations in the genes of interest will only affect the embryos, not the adults. Therefore, we minimise the number of adults born with potentially detrimental mutations.
An exception is the use of adult mice to study reprogramming to pluripotency. To understand how adult cells become reprogrammed to a pluripotent state in the physiological context of the tissue we need to use adults. Mice are the ideal model system as the genetic tools to study pluripotency *in vivo* are already available. We will minimise suffering by first performing experiments in organoids (*in vitro* models of an organ), *an*d only inducing pluripotent gene expression in adult mice for validation of the *in vitro* findings.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will induce genetic modifications only the tissue of interest and during a specific time frame. This will allow us to restrict potential harmful phenotypes in space (tissue of interest) and time (stage of interest).
Pain caused by surgery will be managed with analgesics.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will consult the 3Rs website to ensure that we are following the most refined procedures.
We will follow the ARRIVE guidelines when performing and reporting animal experiments.
We will follow P. Workman et al, British Journal of Cancer, 2010 "Guidelines for the welfare and use of animals in cancer research" in experiments that lead to tumour formation.
We will follow K.H. Diehl et al, Journal of Applied Toxicology, 2001 "A good practice guide to the administration of substances and removal of blood, including routes and volumes" when substances are administered.
We will follow Home Office efficient breeding of animals guidelines:
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Throughout the course of this project we will stay informed about advances in the 3Rs. Our named information officer provides us with up to date information on 3Rs research and training opportunities. We will also maintain a close interaction with animal technicians. They attend multiple meetings and courses, and thus represent an invaluable source of information. In addition, given their experience and expertise on animal research they are uniquely poised to identify ways of improving the welfare of animals. The implementation of any welfare advances will be done with the help and support of the veterinarian and animal care staff.

Home Office

NON-TECHNICAL SUMMARY

198. To Investigate Switching Genes Off and On In Haemopoiesis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Thalassemia, Haemoglobin, Genome organisation, Genomics

Animal types	Life stages
Mice	adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The human genome contains ~20,000 genes, which code for the proteins that constitute the cells that comprise our bodies. Only a subset of the full set of genes are active in any given cell type. For example, the genes coding for the keratin protein in human hair and nails or those coding for haemoglobin in red blood cells are present in every cell, yet normally only active in their appropriate cell type. Abnormal expression of genes in inappropriate cell types is a major cause of human genetic disease and cancers. Although the sequence of the human genome was deciphered in 2003, the mechanisms by which genes are appropriately activated and silenced are poorly understood. This project aims to understand the general principles of how genes are turned off and on in each cell type and developmental stage, how this goes wrong in human disease and how it may be corrected. The aims include determining how genes are activated, silenced and how chromosomal movements influence gene activity in health and disease. We will use the alpha-globin genes as a well characterised example to test hypotheses about gene regulation that will be widely applicable to many diseases.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The majority of human genetic and acquired disease arises from abnormal gene expression, with genes either becoming active when they should be silent or vice versa. Our current understanding of the mechanisms by which genes are expressed and repressed, and how this may be manipulated for therapeutic purposes, is rudimentary at best. The aim of this project is to establish a comprehensive model of how this fundamental process in cell biology and biomedicine occurs within its normal genetic environment (we seek to introduce only subtle genetic modifications so as not to confound our results). We anticipate this will continue to provide direct insight into the mechanisms underlying human disease.

What outputs do you think you will see at the end of this project?

The disorders of the globin genes comprise the commonest single genes disorders worldwide and are responsible for a huge burden of morbidity and mortality, these include diseases such as thalassaemia and sickle cell disease. Although their genetic basis is understood, there are still tens of thousands of cases requiring long term treatments which are still unsatisfactory. To improve treatments of both acquired and inherited disorders we need to understand more fully how these genes are normally regulated. The work to be carried out under the authority of this licence is aimed at using the knowledge obtained from our understanding of gene expression to modify human genetic diseases. Investigating the transcription factors, regulatory environment and epigenetic control of globin-gene expression will enable exact understanding of how globin-genes are activated in these cells. We will generate new information, which will be published in leading journals, detailing how the globin genes are regulated and how this may be manipulated for the benefit of human health. We will also generate regents including genome editing reagents and small molecules that will alter expression of the genes and form the basis of novel treatments for thalassemia and sickle cell disease.

Who or what will benefit from these outputs, and how?

In the short term our findings will allow biomedical scientists to better understand how genes are regulated and how they may be manipulated for the benefit of human health. In addition, our approaches may lead to better diagnosis for atypical forms of thalassemia. In the medium term the disease models we generate will facilitate drug discovery via small molecule screening in culture systems. In the longer term we anticipate that this knowledge will benefit a large number of patients with a disorder of haemoglobin and also a wider group of patients, with a range of disorders, who would benefit from interventions to modulate gene expression.

How will you look to maximise the outputs of this work?

We will disseminate our findings at scientific meetings and by publication in leading peer reviewed journals. Where appropriate we will approach commercial partners to develop novel therapies for the haemoglobinopathies.

Species and numbers of animals expected to be used

- Mice: 5000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Effects of disruption of a normal gene or its regulatory sequences may vary in early, middle or late development and also in different tissues. To study the interactions between different factors during different stages of development and in adulthood a whole animal model is needed. Although zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes, a mammalian model still remains necessary in order to fully understand the effects of many human genes and their disease-associated mutants and other complex physiological systems that only mammals share. Cell lines, differentiated mouse embryonic stem cells and human patient samples will used wherever possible but for much of this work mouse models will be essential.

Typically, what will be done to an animal used in your project?

Typically, mice will be bred and killed by a Schedule 1 method and material such as liver, spleen, bone marrow and peripheral blood will be collected for ex vivo culture and downstream analyses. Some mice may be mated and killed by a Schedule 1 method so that embryos at different developmental stages may be collected for analysis. In approximately 20% of mice, substances will be administered to induce erythropoiesis, to induce gene recombination and/or label cells prior to Schedule 1 killing and collection of biological samples and embryos.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority (we would expect >80%) of the mice in this project should not exceed the mild severity limit but adverse effects that arise occasionally in new GM mice are unpredictable. These effects may be as a result the genetic material introduced, its site of insertion or level of gene expression and although rare are unpredictable. Embryonic mortality may occur and there may be unpredictable effects caused by interference with expression of normal genes or inappropriate expression of the transgene.
New models will be closely inspected for harmful phenotypes, if these arise we will seek advice from the NACWO and /or vet.
In some cases, there will be embryonic lethality (before day 13.5) as a result of the loss of function of the gene of interest.
The administration of substances in this project to disrupt steady state haematopoiesis should only have temporary effects on blood production lineages with full recovery. Thus there should only be mild effects on the overall wellbeing of the animals with a temporary reduction in general activity.

Adverse effects as a result of induction for Cre recombinase are rare but will depend on the nature of the genetic modification and the dose of substance administered. Animals exhibiting any unexpected harmful phenotypes will be killed, or in the case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector.
All animals in this project will be carefully inspected and monitored closely to ensure that they do not exceed the moderate severity limit.
Any animal showing a severity that is likely to exceed this level will be humanely killed unless it is of particular scientific interest in which case advice from the Home Office Inspector will be sought.
Ear notching should involve only slight and transient pain, and no healing problems.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

80% of mice would be expected to experience mild severity
20% of mice would be expected to experience moderate severity

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Effects of disruption of a normal gene or its regulatory sequences may vary in early, middle or late development or in maturity and also in different tissues. To study the interactions between different factors during different stages of development and in adulthood a whole animal model is needed. Although zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes, a mammalian model still remains necessary in order to fully understand the effects of many human genes and their disease-associated mutants and other complex physiological systems that only mammals share. Cell lines, differentiated mouse embryonic stem cells and human patient samples will used wherever possible but for much of this work mouse models will be essential.

Which non-animal alternatives did you consider for use in this project?

Where possible we will employ ex vivo culture of blood from patients and healthy individuals and immortalised human erythroid cells. We have also developed a system of obtaining erythroid cells by in vitro culture of pre-existing mouse ES cells in specific conditions, which has already replaced ~20 lines of mice in our laboratory. This would enable us to apply the in vitro culture system more widely within our institute and more broadly within the university and replace many further lines of mice and more broadly within the university and replace many further lines of mice.

Why were they not suitable?

The culture of human erythroblasts is limited by variation between individuals and the system is limited in only yielding erythroblasts from the pro-erythroblast stage onwards and these stages are limited in numbers. Also, this system cannot accurately recapitulate embryonic erythropoiesis, which we will need to study to achieve our aims.
The system of culturing mouse ES cells to become erythroblasts is extremely useful and we currently use this to test our models, however, this system offers relatively low numbers of cells, it is not possible to accurately stage the cells as they do not express characteristic cell surface markers and they also show an embryonic program of gene expression that does not mature into an adult programme. As stated above we are working to resolve these issues to allow this system to be expanded further.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

Based on the number of mice used in our expiring PPL and our planned experiments, taking into account our analysis pipelines and power calculations.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Improvements have been made in the experimental techniques used to analyse animal material in our projects, fewer and fewer cells can now be used to gain more information. The fetal liver culture system is now being used more widely and continual improvements are being made to expand the number of cells that can be used for downstream analysis.
Further optimisation of this system for particular project can be summarised as follows:

- Selection stem cells prior to culture results in a higher yield of progenitor cells and increases cell viability, reducing need to "pool" individual livers to obtain replicates and thus reducing animal use.

- A recovery step was added for progenitor cells before differentiation ex vivo. This results in more robust and reproducible differentiation. Reducing the need for replicates and therefore additional animals.

- Optimisation of ChIP-seq assays to work on 5x10^6 cells (originally 10x10^6), significantly reducing animal use by providing several technical replicates per biological replicate.

  We have also optimised a newly published method for mapping the locations of chromatin binding proteins and histone modifications genome-wide. Where we have previously relied on ChIP-seq, which required approximately 10 million cells per sample/time point, the new method (called CUT&RUN) requires only 100,000 cells and therefore allows us to carry out many more experiments from the same cellular material. This allows results in fewer mice being used.
  Through our collaboration with a commercial partner we are working to minimise the number of fetal liver cells required. Currently this stands at 50,000 cells per assay. This now means that a single fetal liver allows 140 compounds to be tested.
  We are constantly trying to improve experimental protocols to reduce the cell numbers (and therefore mice) that are required to give us the information we need.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding colonies will be maintained at the lowest possible levels and any excess mice will be made available to other users.
Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers.
We will analyse as many tissues as possible from each mouse.
We will only breed mice as required.
In order to minimise the effects of genetic drift we will only breed for up to ten generations before rederiving lines. Cryo-preservation will be by Sperm freezing wherever possible at an early stage in the breeding process. Embryo freezing will be used only when sperm freezing is not appropriate (e.g. homozygote lines). Any necessary rederivations will be performed by the in house core transgenic service.
All steps in every process will be carefully monitored to minimise numbers.
Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers.
Blood and tissue samples will be shared across several groups working on this project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse models with perturbations of the alpha-globin locus used to investigate gene expression are based on a locus that encodes a terminally regulated structural protein and as such disruption of expression does not

lead to cell fate changes or neoplasms minimising any suffering or distress caused to the mice. In addition, disruption of the locus is asymptomatic when in the heterozygous state and where possible heterozygous experimental animals will be used. Tagging of transcription factors will be checked by in vitro differentiation of mES cells to ensure it does not interfere with normal gene function before generating mouse lines.
In cases where the a protein degradation system is used to acutely deplete TFs, pregnant mothers will be protected from adverse effects of drug induction by paternal inheritance of the protein that effects the protein degradation. Acute depletion of transcription factors is preferable to creating constitutive knock-outs as these models often die early in development from adverse effects in non-erythroid tissues, potentially masking any scientifically informative erythroid phenotype.
We will use the least invasive methods to administer the substances described in this project. We will administer tamoxifen by oral gavage unless this does not yield sufficient recombination in a tissue of interest, in which case injection may be necessary. When administering substances to pregnant females for metabolic labelling we have previously used intraperitoneal injection to achieve the fastest possible delivery of substance to the embryos during the short delivery window (approximately 30m). To minimize the risk of inadvertent injection into the uterus, intraperitoneal injection of pregnant females will be carried out only by the most experienced PILs. The female will be positioned with her head down so that the abdominal contents move away from the injection site. The substances themselves are generally innocuous and are extremely unlikely to cause any distress to mice. In cases where induction of mild anaemia is unavoidable to allow analysis of gene expression this will be kept to a minimum, mice will be monitored and humane endpoints applied appropriately.

Why can’t you use animals that are less sentient?

Although zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes particularly at the early stages of research, a mammalian model still remains necessary in order to fully understand the effects of many human genes and their disease-associated mutants and other complex physiological systems that mammals share. Where possible we do use embryonic forms, however, for many experiments we need to analyse adult erythropoiesis in liver or spleen cells.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Wherever possible constructs and/or manipulated embryonic stem cells will be produced and tested in an in vitro system before going on to produce new GM lines.
We continue to have a high level of enrichment in our cages to promote natural behaviours and improve general welfare. All cages contain sizzle nest and polycarbonate tunnels as standard: singly housed stud males are given wooden chew toys to prevent boredom and our breeding pairs and pregnant females are also offered mouse smart houses and nestlets.
We continue to use tunnel and /or cupping as a means of handling of mice as promoted by the NCRs. In our experience these mice do get used to this very quickly and are much calmer and easier to handle. We would very much like to see this method being implemented on a wider scale wherever appropriate.
Use of our in vitro fetal liver culture system has increased as an alternative to drug treatment of adult mice in order to obtain haematopoietic cells for analysis. Where drug treatment is necessary we will use best practice with the single use needle procedure.

Where possible we add a companion female to singly caged pregnant females and we have adopted the policies recommended by the Home Office and make sure no aged mice are maintained or used for breeding.
We continue to cryopreserve lines at an early stage in the breeding programme to ensure the line is preserved at an early generation. If it is necessary to use a line for a prolonged period we will rederive before reaching 10 generations to minimise the effects of genetic drift.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the PREPARE and ARRIVE guidelines and the LASA "Guidance on Aseptic Surgery" for advice on best practise when administering substances and also guidance available on the NC3Rs website.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I will routinely attend NC3R's meetings, animal welfare meetings where best practices are discussed. The group will keep abreast of the latest literature and read the 3R's newsletters and adopt new methodologies and procedures as they arise.

Home Office

NON-TECHNICAL SUMMARY

199. TOWARDS PREVENTION AND CURE OF HIV INFECTION BY ACTIVE IMMUNIZATION

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

HIV, Virus infections, Preventive vaccination, Therapeutic vaccination

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of the project is to make significant contributions towards the development of a safe and

effective HIV vaccine focusing mainly, but not exclusively on induction of protective T-cell responses.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In 2018, 1.7 million people were newly infected with HIV, 770,000 died of AIDS-related illnesses and an estimated 37.9 million people were living with HIV-1 (PLWH), of whom approximately half received antiretroviral treatment (ART). Prevention largely focuses on behavioural and biomedical interventions such as male circumcision, provision of ART as pre- and post-exposure prophylaxis and mother-to-child transmission, while HIV cure concentrates on long-acting injectable drugs, slow-release implantation devises and vaginal rings. For PLWH, ART saves lives, but it does not cure HIV and has to be maintained for life, which magnifies side effects, stigma and significant economic and logistical challenges particularly in low- and middle-income countries. Given the challenges for widespread implementation of the biomedical measures, an effective vaccine has always been and remains the best solution and likely a key component of any strategy for ending the HIV pandemic.

Although the HIV-1 vaccine field has recently focused almost exclusively on B-cell analysis and induction/introduction of broadly neutralizing antibodies (bnAbs), there is a renewed interest in T-cell vaccines. In humans, there is a large body of data implicating a protective role of T-cells against HIV-1, which was demonstrated directly in an experimental infection of macaques by simian immunodeficiency virus (SIV). Clearly, T-cell responses impose a selective pressure on the virus and their antiviral impact should be harnessed for vaccine protection if only to complement antibodies. The trick is not to induce just any responses, but well targeted protective killer T cells.

What outputs do you think you will see at the end of this project?

The overall aim of the proposed program is to make significant contributions towards the development of a safe and effective HIV vaccine mainly through induction of protective T-cell responses. Early HIV vaccine development is an important, but relatively high-risk area of research, which can be only sustained by the academic sector; following the initial few failed vaccine efficacy trials, big Pharma has reduced its interest, but would become re-engaged if a new strategy showed a real promise of efficacy, particularly if the candidate vaccine was well suited for HIV cure. By the end of this project, we shall extend our understanding of the host-pathogen interactions, gather novel information on protective responses against pathogens and their induction, develop new skills and expand the toolbox of vaccinology.

Who or what will benefit from these outputs, and how?

HIV vaccine will transform lives of millions of people particular those living in low- and middle-income countries. Development of vaccines against human diseases has many features in common.

Demonstration that focusing vaccine-elicited T-cell responses on functionally conserved regions of the proteome can control HIV-1 would encourage similar strategies for fighting other human pathogens. Through thorough investigations of HIV-1 biology and vaccine development, HIV research has significantly contributed to almost every other field of biomedical research. With the renewed focus on infectious diseases, pandemics and demonstrated societal impact pathogens can have on communities and state and even global economies, the time has never been better to extend our understanding of the host-pathogen interactions, gather novel information on protective responses against pathogens and their induction, develop new skills and expand the toolbox of human vaccinology.

How will you look to maximise the outputs of this work?

All results will be presented at international meetings and published in peer-reviewed scientific journals. The proposed research will advance understanding of crucial attributes of protective T-cells against HIV-1 and their induction. Development of vaccines against difficult, highly variable human pathogens is challenging, but the strategy and tools are common; success in controlling the AIDS virus will likely provide a new powerful tool with broader applications for improving public health. Therefore, academics developing prophylactic and therapeutic vaccines against infectious diseases and cancer will benefit from our research. Similar strategies apply to prevention and treatment of animal infections.
Eventually, the HIV programme will contribute to the reduction of the social and economic burden caused by HIV-1/AIDS, a poverty-related disease, by accelerating the development of an effective, safe, accessible, suitable and affordable preventive vaccine against HIV and other viral infection.

Species and numbers of animals expected to be used

- Mice: 5300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the lowest vertebrate group in the evolutionary tree for which suitable models of immune responses in humans and reagents are available. Adult animals are sufficient to answer all scientific questions in this model before progressing to humans.

Typically, what will be done to an animal used in your project?

An animal is typically administered substances by injections. The route is dependent on the vaccine platform, but is usually either intramuscular or subcutaneous, though other routes may sometimes be required. Experiments can last between three weeks to a year though a typical experiment last one month when a single-prime and single-boost regimen is used.

The animals are always killed by a schedule 1 method for the harvesting of the spleen from which splenocytes are purified for assays.

What are the expected impacts and/or adverse effects for the animals during your project?

Localised short-term pain at the sites of immunisation and blood sampling. In our over 20 years of using the mouse model, we have very rarely encountered adverse effects during the projects as the vectors we use have a long-proven track record for safety.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

I expect 98% of animals to be returned as Mild and 2% as Moderate.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Vaccines have to be tested in living body: The procedures described in this PPL cannot be done using cells, isolated organs or animals kept under general anaesthesia. The vaccine-driven expansion of immune responses and optimization of delivery requires injection of candidate vaccines into a living body. The use of live animals is needed to study tissue distribution/homing of vaccine-generated T cells; there are no alternatives.
Human studies. Every Good Manufacturing Practice vaccine batch is tested for immunogenicity in mice prior to use in humans to confirm that the vaccine is fit for purpose. The batch testing of candidate human vaccines is fully and adequately covered by Protocol 1: we inject animals i.m. by the candidate vaccine, kill the mice 1 week later and assess immunopotency on isolated splenocytes. These are small-batch-size Investigational Medicinal Products for phase 1 and phase 2 Experimental Medicine trials rather than licensed vaccines mass produced by Pharma.

Which non-animal alternatives did you consider for use in this project?

Some very specific questions can be answered using cultured cells, for example defining the small parts of microbes recognized by the immune responses can be performed by expanding specific T-cell clones in culture. But the responses/the immune cells still need to be generated in vivo.

Why were they not suitable?

Some very specific questions can be answered using cultured cells, for example defining the small parts of microbes recognized by the immune responses can be performed by expanding specific T-cell clones in culture. But the responses/immune cells, still need to be generated in vivo.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

Group sizes of animals of individual experiments will vary. For new previously untested vaccines, vaccine combinations and vaccination protocols, the number of animals per group for assessing T-cell immunogenicity is empirically n=5. For challenge experiments and longer-term protocols, this number may be increased empirically. In cases where the vaccine effect or anticipated improvement in a given assay are known from previous results, the number of animals used in a group will be calculated in consultation with a statistician. This will be done on an experiment-by-experiment basis.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

For induction of cell-mediated immunity by vaccination, unvaccinated control groups are typically not included as the background peptide responses in naïve mice are well established and minimal. Control groups will be needed ONLY for experiments determining protection against the surrogate virus challenge.
In an effort to reduce numbers of mice used for vaccination studies, we have now implemented blood sampling as our standard method of immune monitoring. This allows us to follow individual mouse responses throughout an immunization experiment and thus obtain data from multiple time points from one mouse. The main limitation is the blood volume/cell numbers recovered from a living animal.
Validated potency tests for clinical trial vaccines will be carried out in groups of 5 BALB/c mice using H2Dd-restricted epitope included in the immunogens. Based on the past variability of the assay, five, four or three animals fulfilling potency criteria give us 98%, 91% or 71% confidence (p=0.05), respectively, that the vaccines are fit for use.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In our experiments, the group sizes of animals will vary. For new previously untested vaccines and vaccine combinations, the number of animals per group for assessing T-cell immunogenicity is empirically n=5. For challenge experiments and longer-term protocols, this number may increase. In cases where the vaccine effect is known from previous results, the number of animals used in a group may decrease and will be calculated in consultation with a statistician to achieve at least 80% power to discriminate between compared groups ($p<0.05$). This will be done on an experiment-by-experiment basis.
The number of mice used in these studies will be kept to a minimum to answer the scientific questions based on statistical calculations and reproducibility. We shall be using inbred mouse strains, which have a homogenous genetic background that decreases variation in the data, we shall be able to use smaller numbers of animals to obtain good data. Furthermore, whenever possible experiments will be designed to overlap and allow use of tissues from the same animals for multiple experiments answering different questions.
Overall, our experiments are very basic and straightforward. I do not immediately foresee any potential for a novel reduction.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This animal license authorizes the use of mice for experimental testing and development of HIV vaccines. These vaccines will be typically administered to animals using the intramuscular route. As described in the protocols, animal suffering will be minimized by establishing and using the minimum dose necessary to achieve desired levels of immune responses, minimizing the number of vaccine injections, using predominantly of the most applicable route of vaccine delivery for humans, which is the intramuscular needle injection, and for peptide and

proteins, employing adjuvants safe and acceptable for use in humans, including newborn babies. All vaccine dosings are carried out aseptically,

Why can't you use animals that are less sentient?

The aim of our research program is to develop efficacious vaccines. As such, we need an animal model with a good immune system that allows us to test if our vaccine candidates are immunogenic over time. Furthermore, we have developed our toolbox in the strains of mice we use, which maximizes the information gained using minimum number of animals. Animal that are terminally anaesthetised are also inappropriate, as we need to study the durability of adaptive immunity. This is simply not possible for a terminally anaesthetised animal.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We use sterile yellow tips inserted onto the syringe needle to control depth of needle insertion into the muscle. This reduces the risk of the needle damaging tissue as well as provides consistency in immunisation. We will continue to ensure that PIL holders are well-trained in the lab using models or teaching aids, where possible, before progressing to work on live animals.
The best means to reduce pain for the animals is to administer the procedure skilfully and, where possible, administer it under general anaesthesia. To this end, the PILs working are this PPL are overseen by an experience senior scientist who endeavours to work together with each less experienced PIL holder until they are signed off for a specific procedure.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow guidance on the administration of substances and aseptic techniques.
We take advice from the NC3Rs as part of our experiment design and planning. We endeavour to the employ the method with the lowest cost in terms of harm/pain to the animal.
We discuss experimental designs and publication requirements with colleagues, peer-reviewers and editors. All PPL and PIL holders attend the termly Animal welfare meeting, where all stakeholders from different groups and institutes share information.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The employing institution has a 3Rs officer who regularly disseminates advances in reduction, replacement, refinement practices with regards to the species of animals we need to use. This information as well as that given at the compulsory termly meetings of the users of the animal facilities is discussed in depth and adopted as standard practices.

Home Office

NON-TECHNICAL SUMMARY

200. Toxicology of Pharmaceuticals

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Toxicology, Safety, Regulatory

Animal types	Life stages
Mice	juvenile, adult, aged, embryo, neonate, pregnant
Rats	juvenile, adult, aged, embryo, neonate, pregnant
Rabbits	adult, pregnant, neonate, embryo, juvenile
Pigs	juvenile
Beagles	juvenile, adult
Cynomolgus macaques	juvenile, adult

| Animal types | Life stages |

Minipigs	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of the project is to evaluate the safety of various types of potential new human drugs when given to test animals. The work is required for new drugs, for the safety of the human volunteers and patients who will take the drugs, and it is designed to meet the requirements of regulatory bodies in Europe and elsewhere, who must agree to the sale and use of drugs. **A retrospective assessment of these aims will be due by 09 April 2026**

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

As well as assuring the safety of human volunteers and patients, the successful conduct of tests will help bring to market those materials which are safe and are subsequently shown to be effective in the treatment or prevention of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework.

What outputs do you think you will see at the end of this project?

Data collected will be information on how animals are affected by potential new medicines. This will include efforts to identify systems within the body or particular organs of the body that may be affected by short term or accumulated exposure to the new medicines. Outputs will include simple measures like changes in behaviour, food consumption, growth rate and weight retention or loss. Samples will commonly be taken, particularly of blood, but also other excretions such as urine to assess any changes over time, as well as to assess how much of a medicine has been absorbed or excreted. Post mortem examination can demonstrate change in function or structure of body organs, including examination at a microscopic level. Some studies will be to check if there is any effect on ability of animals to breed, or any effect on the development of the young in the uterus.

The data will be collected to the standards required by government regulators in the UK, Europe and elsewhere, for identifying and excluding inappropriate medicines due to safety concerns, and enabling further development of successful medicines.

Improved methods of conduct of specific data collection processes may be developed during the course of the project.

Who or what will benefit from these outputs, and how?

Our clients, typically commercial drug companies, will benefit from the provision of high quality data. This will help them in their work to develop new and better medicines, to discontinue development of inappropriate medicines or to understand and manage the risks of new medicines given to people. Work on this project may also provide data to inform ongoing human clinical trials.

Enabling development of successful medicines will benefit society through diagnosis, treatment or prevention of disease.
Identification of adverse effects can prevent future harms to human volunteers or patients by resulting changes to medicine development programmes.
The wider scientific community may benefit from publication of refined approaches to animal use.

How will you look to maximise the outputs of this work?

Our organisation has colleagues with extensive experience of such work in different parts of the world. Collaborations and information exchange with others within the organisation helps to identify and spread information on successful and unsuccessful approaches.
Collaboration with clients (knowledge gained on products).
On-going collaborations with NC3Rs on various aspects of regulatory safety studies, over many years.
Presenting outputs at scientific conferences and contributing to publications in the scientific literature where relevant.

Species and numbers of animals expected to be used

- Mice: 20000
- Rats: 45000
- Rabbits: 4000
- Beagles: 4000
- Cynomolgus macaques: 3500
- Minipigs: 900
- Pigs: 30

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Many scientific studies have been conducted to demonstrate that the types of animals to be used in the project will provide results which reflect the likely effects in humans. The way in which each new medicine works in the body will be known, and the animal type(s) to be used will be chosen based on an understanding that the medicine will work in a similar way.
The stage of life of the animals to be tested reflects the age/stage of life of people who would receive the

medicines.
Another big advantage of using the listed animal types is that these animal types may be recommended by specific guidelines on how to do this work, and the results of tests are known to be acceptable to the government agencies responsible for authorising use of the medicines in human volunteers and patients. Development of new medicines cannot currently be achieved without this approval by government agencies in the UK, elsewhere in Europe and in other parts of the world.

Typically, what will be done to an animal used in your project?

Animals will be given a potential new human medicine by the same method that people would be exposed to them - most commonly by mouth, but may be by injection, application to the skin, or by inhalation. Inhalation of materials generally requires that animals are accustomed to close restraint in a purpose-made device while breathing the medicine, and/or wear a mask while breathing the medicine. If applying medicines to the skin, some form of covering or temporary restraint is required, to stop the animal or cage-mates interfering with it. Dosing of the medicines is for at least as long as people would be asked to take a medicine. A small number of studies involve giving the medicine to rodents for an estimate of their lifetime, to check if it might cause cancer. A small number of studies involve surgery, to allow dosing of medicines intravenously for an extended period daily, or continuously. Surgery may also be conducted to take tissue samples, to assess a change in effect over time or to allow collection of information such as ECGs without restraint of animals. All surgery is conducted under anaesthesia and with use of post-surgical pain relief, under veterinary guidance.
Other samples such as blood samples are commonly taken to assess any effect and/or to assess how much of the medicine is absorbed. Behavioural tests may be conducted to check for effects. Other specific examinations may be undertaken, including examination of the eyes. Some studies are to assess if the material has an effect on the unborn, or on the development of young animals.
Animals will usually be used once only, and then will be humanely killed to check for effects in the body, including by examining the tissues microscopically.

What are the expected impacts and/or adverse effects for the animals during your project?

The process of dosing animals or taking samples can cause a degree of discomfort during conduct, particularly if animals have to be restrained to enable the work. Behaviour and health may be affected by the materials being given, and reduced health can be measured, e.g. by reduction in food consumption, weight loss, changes in blood results. Some studies may have effects on the ability to breed or on development of the young. In lifetime studies, adverse effects are usually those seen in ageing animals, such as reducing function of the body's organs, resulting in reducing quality of life over time.
Surgery can cause some discomfort in the immediate post-surgical period, but this is prevented or minimised by use of appropriate anaesthetics drugs and pain relief.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The vast majority of animals which may experience harms as described above are expected to be considered as mild severity. This includes 85% or more rodents and 70% or more non-rodents expected to be used in the project. It is expected that about 80% of animals to be used in the project will be rodents.

Most other animals may experience harms categorised as moderate. Severe outcomes are not anticipated in most studies; about 1% to 1.5% of rodents may be used in a severe protocol for testing of anti-cancer medicines intended for use in patients with late-stage cancer. Some of these animals may experience harms which are categorised as severe, including lack of appetite, significant weight loss, diarrhoea; such animals would be

humanely killed. Some animals may experience no apparent harms.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Rehomed

A retrospective assessment of these predicted harms will be due by 09 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Non-animal methods are routinely used in some aspects of the development programme for new human drugs, but they are currently not able to sufficiently predict effects on whole body systems or to provide information on how much of a medicine is absorbed. It is not currently possible to acquire all of the information on how the body systems such as the heart, brain, liver and kidneys may be affected by new medicines, without using animals. This information is essential, to protect human volunteers and patients. The protocols described in this project are conducted according to internationally-agreed guidelines, and are expected to be performed before government authorities will authorise giving new experimental medicines to people.

Which non-animal alternatives did you consider for use in this project?

The organisation does conduct various non-animal tests as part of the development programme for new medicines, but as noted above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes.

Why were they not suitable?

There currently remains general scientific agreement, and agreement of government regulators, that to protect human volunteers and patients, non-animal alternatives do not, as yet, provide enough information to replace all animal studies.

A retrospective assessment of replacement will be due by 09 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated need for use to a similar extent.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

There are various guidelines issued by government regulators on how to conduct the various study types described in the licence. While giving general information on study design, most of these do not give specific information on the numbers of animals to be used in a study. Sufficient, but minimum numbers are expected to be selected. In the case of reproduction toxicity studies, specific information on animal numbers is provided. Where there is no definitive regulatory guidance on numbers of animals, the applicant and colleagues will use their extensive experience of related programmes, taking account of statistical significance and scientific advice to use sufficient animals for studies to provide robust results, known to be acceptable to government regulators. In general, longer-term studies will use larger group sizes of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies will be used to investigate the potential of new designs or processes to improve outcomes, before being used in larger numbers of animals. Initial screening studies, using small numbers of animals, are designed to identify and eliminate materials with undesirable results, and so reduce the numbers of animal which are then used in the studies required by government regulators. Short-term studies are conducted, and results assessed, before starting longer-terms studies in the same programme, to ensure the need for the follow-on study, and to help maximise the study design opportunities.
A retrospective assessment of reduction will be due by 09 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Dosing of potential new medicines is by the same way as they would be given to people; most commonly by mouth, but including various injection methods, dosing by inhalation and by application to the skin. The methods

used are generally very well established and commonly used by experienced staff at the establishment. Volumes of drugs to be given are in line with published guidance on minimising discomfort, and/or are known to cause minimal discomfort based on extensive experience at the site. Any novel volumes would be tested to confirm lack of discomfort before further use; he Animal Welfare and Ethical Review Body must approve any such requests. The amount of a drug, and the amount of time dosing is continued, depend on how much and how long people might be expected to take a drug.
Blood sampling is a common need. We follow published guidance on methods and suitable volumes which can be taken while minimising harms to animals.
Restraint or confinement of animals is occasionally needed to allow conduct of dosing or sampling processes. Methods used are those with which staff have extensive experience, and the duration of time is minimised wherever possible while allowing completion of the process so that tasks do not generally have to be repeated.
Surgery is conducted with expert veterinary involvement in the creation of suitable regimes for anaesthesia and post-surgical pain relief.

Why can't you use animals that are less sentient?

The species used are selected based on known standards of outcome which will answer the scientific questions. They are also known industry and regulatory standards which will meet expectations of government regulators internationally. The particular species chosen for a programme may also allow comparison with other data which as been generated using the same species with the same medicine, or similar medicines, to assess which might be the best for people to take.
Response to tests is assessed over a time period which would make continued anaesthesia impractical in almost all cases, and would interfere with the outcome in some circumstances.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinement of on-going procedures is commonly discussed and explored within the animal technical, veterinary and scientific groups, and also as and when any concerns are identified; for example additional assessments may be included based on initial outcomes.
The surgery and anaesthesia/pain relief protocols used in the programme undergo regular and routine assessment and refinement to improve outcomes. Habituation of animals to restraint is a routine process, and the schedule can be amended in response to outcomes for individual animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Dose volume and blood volume limits agreed with the animal welfare and ethical review body are based on the 2001 publication of Diehl *et al*: A good practice guide to the administration of substances and removal of blood, including routes and volumes.
Welfare end-points are developed in general line with publications on the topic, including the NC3Rs document from 2010 on dose level selection for regulatory toxicology studies.
Non-human primate housing is in compliance with the NC3Rs document on this topic from 2017.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Both our clients and our colleagues working in the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which

propose refined approaches to conduct of work.

A retrospective assessment of refinement will be due by 09 April 2026
The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

201.Tracking Salmonid Smolts and adults to establish in river survival rates and coastal movements

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (d) Protection of the natural environment in the interests of the health or welfare of man or animals (e)
- Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work

Key words

Sea trout, Salmon, Acoustic tags, Marine distribution, Survival

Animal types	Life stages
Other fish species	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To better understand salmon and sea trout migration and provide data on in river and marine movements, including the potential and actual effect of man-made structures in coastal and river environments

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Fish which migrate into freshwater to spawn, such as salmonids, may have to migrate past coastal and in-river developments to complete their life cycle. Developments such as weirs, barrages, tidal lagoons or major abstractions have the potential to impact on survival of both juvenile and adult stages.
This can be particularly important for species such as Atlantic Salmon (*Salmom salar L.*), sea trout (*Salmo trutta L.*) or twaite shad (*Alosa fallax*), which spawn multiple times and may therefore be subject to cumulative impacts. The lack of data on migration patterns of these species, and hence potential impact, has impaired the ability of developers to assess impacts and propose suitable mitigation, compromising marine licence applications and potentially putting the fish populations at risk.
This project will track up to 300 sea trout smolts, up to 200 sea trout adults, and up to 100 salmon smolts tagged with acoustic tags. The tags regularly emit a coded acoustic pulse which can be detected and decoded by fixed passive receivers, enabling individual movements to be followed. Fish will be tagged in freshwater, and their migration to sea followed, to develop quantitative survival, migration and availability data for a specific coastal location. It will also look at the impact of a major in river structure at head of tide. The project will provide specific data for an important coastal development area, and contribute to the development of a wider understanding of in river survival, coastal and marine phase movements, and distribution of sea trout.

What outputs do you think you will see at the end of this project?

1. Quantitative data on distribution and residence times of salmon and sea trout in the immediate study area.
2. Qualitative data on coastal distribution and migration paths in a wider area.
3. River and sea survival / return data for sea trout
4. Evidence describing migration past a structure at the tidal interface (some limited data exists already at this site, but this study will significantly improve knowledge).

A final project report will be published and made publicly available. The more important results will be published in peer reviewed journals.

Who or what will benefit from these outputs, and how?

Lack of data on key marine species, including migratory fish, is recognised as a strategic information gap by both regulators and industry (see the ORJIP report, 2017).
Developers and regulators will be provided with valuable data as the study progresses; migration data and

survival would be analysed and reported on an annual basis. Information specific to the area will benefit local regulation and inform evaluation of current and future development proposals. The information will therefore provide both short and long term value. Results describing migration and behaviour patterns in inshore areas will have wider utility and will benefit assessments by regulators elsewhere in the UK. The value regulators place on this data is reflected in financial commitments (tag and receiver purchase) to help support the work.
Local angling associations are keen that evidence is developed to ensure that both the fish population and fishery are protected through the regulatory process for marine developments. They have provided/are providing practical assistance with our fieldwork, including fish capture.
We would also expect to provide information regularly during the course of the project to the wider scientific community through conference papers and publications.

How will you look to maximise the outputs of this work?

We expect to produce reports, conference papers and peer reviewed publication.
We are already working closely and collaborating with UK regulators, governing bodies, conservation authorities and Trusts.

Species and numbers of animals expected to be used

- : Up to 600 fish

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Sea trout migrate to sea as smolts, returning to spawn as adults. During these migrations they may be killed or their migration success compromised by various natural and human activities, including natural factors such as predation and disease, and man-made problems such as the presence of barriers (weirs and barrages), pollution, marine and in-river developments.
In the natural state, sea trout normally spawn multiple times, unlike salmon, where only a small percentage survive spawning. As such the impact of human activities can be multiplied through their lives, as losses may compound with each migratory phase.
At the current time sea trout populations in many parts of the UK are at historically low levels. However much less is known about sea trout than salmon, particularly their movements in the marine environment. This study aims to look at sea trout survival rates at different life stages, and will focus on the impact of specific man made developments and development proposals. This is important information for managing and protecting sea trout populations.

Typically, what will be done to an animal used in your project?

Fish will be captured using nets or traps specifically designed to avoid damage. They will be anaesthetised and tagged with an acoustic tag through an incision approximately 1cm long (or less). The incision will be closed with a dissolvable suture and covered with a suitable covering to initially prevent the wound from infection during initial healing.
Juvenile fish will then be transferred to a well aerated recovery tank and monitored for normal behaviour (holding their position and actively swimming). Adult fish will be held facing into the water stream until they are able to hold position and actively swim upstream.

Once the fish are recovered from anaesthesia they will be released to continue normal lives.

What are the expected impacts and/or adverse effects for the animals during your project?

Experience has shown that fish rapidly recover from anaesthesia and surgery and are not expected to suffer any lasting long term harm as a result of the procedures under this protocol being carried out.
The procedures carried out in these protocols will be done under general anaesthetic and therefore fish will be subjected to no more than mild stress as a result of capture and handling. There may be some mild post-operative discomfort, but experience of staff carrying out the work will ensure that juvenile fish are only released once they are behaving in a normal manner within the release tank, or for adults, when they are able to swim upstream against the flow.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect the severity to be moderate for all fish tagged. The tagging approach is intended to minimise discomfort or damage to the fish.
What will happen to animals at the end of this project?

- Set free

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The project aims are to look at the behaviour and distribution of Atlantic salmon (*Salmo salar L.*) and sea trout (*Salmo trutta L.*) in the wild in order to gain information to manage and protect the species in the context of specific development areas. There are no practical alternatives to generate this data.

Which non-animal alternatives did you consider for use in this project?

Theoretical modelling has already been utilised to look at potential distribution and movement in the study area. This has identified the species most at risk from developments and these are the subject of these studies.

Why were they not suitable?

In the absence of migration studies and actual movement data for sea trout in the study area, there is no basis to validate these models and their predictions.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may

include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We need to use sufficient fish to provide robust estimates of survival at different life stages to inform models which can then be used to predict impacts without the requirement for further experiments using live fish. Sea trout life cycles are more complex with multiple spawning returns common and a sea complex age structure. For this reason we aim to tag more sea trout (up to 500 in total). For salmon, where we are tagging smolts and looking at emigration through a more limited area, we would aim to tag up to 100 fish, over two years. Our initial estimate is therefore that this *could* require tagging up to 600 fish to generate robust data.
In practice the key factor in determining the number of fish required will be survival at different life stages. The numbers given above are maximum numbers and design and actual numbers will be set on a 'pilot study' basis and informed by past results as the project evolves.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have undertaken a small pilot study to provide proof of concept, develop sites, skills and procedures and provide some initial data to inform year 1 tagging requirements. Each phase of work will be reviewed to inform estimates of required sample sizes at the next stage and to minimise unnecessary capture and tagging.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot study as above; developing computer models which can be used in subsequent studies to reduce requirements for similar work.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Both salmon and sea trout are economically important for recreational fisheries; they are also important conservation species, widely recognised as a measure of the health of river systems. Marine developments are routinely required to demonstrate that they will not impact salmonids, yet the data to do this are highly limited. For tidal developments, modelling has demonstrated that sea trout are a particular concern.
The capture and tagging of methods we are using with acoustic and radio tags are well established. The methods we are using are designed to allow the fish to return as rapidly as possible to normal behaviour with minimal long term effects.

Why can’t you use animals that are less sentient?

Our objective is to understand observe behaviour and distribution of sea trout and salmon under natural conditions, including at sea. This cannot be achieved by other means.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Capture method

The trap we are using for adults has a large holding area and will be fished at least twice a day. For smolts the Fyke nets we are using a design with a large soft meshed cod end designed to minimise damage to the fish. We will remove smolts from the nets at regular intervals during the night.

Choice of tags

We are using the smallest tags available consistent with the objectives of the project, including tag life and tracking in the marine environment. The tags we are using are specifically designed by the supplier (VEMCO) for work with the species and life stages we are using. They are tough and smooth to minimise any issues if ingested by a predator.

Tagging and recovery procedures

The anaesthesia technique we are using for both smolts and adults ensures water circulation throughout the procedure.
When tagging during dark hours (smolts) light will be kept to a minimum to reduce stress. Aseptic surgery techniques and single use scalpel blades and suture needles will minimise risk of infections.
Each incision will be covered with a suitable temporary wound barrier to provide a temporary barrier, reducing discomfort and providing protection from infection. Sutures will be checked prior to transfer into recovery and holding tanks.
Smolts will be monitored and only be released in groups when exhibiting normal swimming behaviours. Adults will only be released when they are able to swim upstream and maintain position against the flow, and will be released into a quiet deep pool where they can shelter in lower flows until ready to swim away.

Smolts will be released in groups to maintain shoaling behaviour.

Humane end-points and limits of severity

If internal damage to organs were to occur during surgery, the fish would not be allowed to recover and would be euthanized by a schedule 1 method.
If fish fail to recover from anaesthesia they will be euthanized by a schedule 1 method

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

There are a number of published studies using these tags and techniques. However methods evolve continuously and we have taken best practice advice from regulators, other scientists, Non-Governmental Organisations working in this field and our NVS. All the above are undertaking current licenced work with these species and our approach and protocols seek to take the best from each, consistent with our objectives.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continually review the literature. We will attend conferences, such as the recent SAMARCH workshop, which brought together salmonid tracking researchers. We will continue to network with others to share and learn from further developments, both as research understanding of the field develops and to improve our

tagging methods to minimise any potential adverse effects. Where appropriate we will update our protocols and methods.

Home Office

NON-TECHNICAL SUMMARY

202. Training in complex surgical procedures

Project duration

1 years 0 months

Project purpose

- (f) Higher education or training for the acquisition, maintenance or improvement of vocational skills.

Key words

No answer provided

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project will train surgeons in advanced, therapeutic, minimally invasive, surgical procedures.
A retrospective assessment of these aims will be due by 05 February 2022

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

In many cases minimally invasive (keyhole) surgical procedures are significantly better for patients than open procedures as they are associated with less post-operative adhesions, less time in hospital, faster recovery, less pain, easier post-operative care and much faster return to active life. Consequently, many new minimally invasive procedures are being developed to replace larger, open procedures - particularly in response to the Governments new screening programmes for bowel cancer and aortic aneurysm among others. These screening programmes are identifying 30-40% more patients requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited. Un- or insufficiently trained use of these new procedures results in unacceptable death rates and long term side effects. We aim to teach surgeons these new, complex procedures, in terminally anaesthetised animals, to ensure rapid competency and safety. These courses will ensure an adequate supply of appropriately trained surgeons who will be able to fulfil the needs of our increasing numbers of patients using new minimally invasive procedures safely and effectively.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project? 51 pigs

and 8 sheep over the course of the licence

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

As all protocols are non-recovery, and animals are given an anaesthetic overdose whilst under anaesthesia, no adverse effects are envisaged. Also, at the end of the procedures, all possible tissue and organs are harvested for use in other studies as well as for use in other training courses.
A retrospective assessment of these predicted harms will be due by 05 February 2022

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

As yet, there are no simulators that truly represent the full physiological state necessary to teach these procedures. Current simulators are unable to replicate the blood and lymph flow of tissues and are also not able to replicate tissue responses to stimuli, muscular activity in bowel, effects of surgery affected by temperature, or tissue changes relative to procedures. We will endeavour to develop better simulators as these courses progress.

A retrospective assessment of replacement will be due by 05 February 2022

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

By carrying out a number of procedures in one animal we can reduce the number needed and, as all animals will be deeply and terminally anaesthetised, there will be no suffering or adverse effects. Using 2 animals per 3 or 6 surgeons depending on the course also reduces the number of animals needed.

A retrospective assessment of reduction will be due by 05 February 2022

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The pig and sheep have been chosen for these courses as we need to represent the same size and physiology as humans, in particular with regard to blood system, lymph system, tissue response and general anatomy. Principally, animals are terminally anaesthetised and therefore insentient throughout.
They are carefully monitored using staff trained, skilled and experienced in ensuring effective prolonged anaesthesia in these species.

A retrospective assessment of refinement will be due by 05 February 2022

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

203.Training in Murine Polio Intraspinal Inoculations

Project duration

5 years 0 months

Project purpose

- (f) Higher education and training

Key words

Training, Polio, Vaccine, Batch release, Research and development

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of the plan of work is to enable a team of prospective and existing practitioners to develop and maintain manual skill in the inoculation of substances into discrete areas of the mouse spinal cord with sufficient accuracy and reliability to satisfy established World Health Organization (WHO) criteria for the polio vaccine safety test using transgenic mice expressing the human poliovirus receptor.
A retrospective assessment of these aims will be due by 27 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Skills gained and maintained as a direct result of the procedures applied in this project will be utilised in the successful conduct of definitive the transgenic mouse polio vaccine safety test (Tgm test). The most immediate benefit of the proposed training programme will be that it will be possible to retain a pool of appropriately qualified and competent practitioners able to perform the Tgm test in-house.
The benefits arising are:

1. Implementation and use of the Tgm test will continue to significantly reduce the use of nonhuman primates for batch-release testing of monovalent poliovirus vaccine bulks both in UK (if still required) and in Europe.

2. The Tgm test is now included in the European Pharmacopoeia (EP) and there have been strong ethical, practical and financial imperatives upon the vaccine manufacturers to take up the test as they are increasingly doing. The lack of an Official Medicines Control Laboratories (OMCL) in Europe adequately qualified and competent to complete the test or comment on its execution would severely compromise the public acceptability of the test within Europe.

3. The WHO will continue to have access to a suitably qualified reference laboratory to which matters of global health significance relating to neurovirulence of poliomyelitis vaccines can be referred.

4. The parameters detected by the Tgm test compared to other possible assays will be better established giving greater confidence in its reliability and meaning for product safety.

5. New strains to be used for live-attenuated oral polio vaccine (OPV) and inactivated polio vaccine (IPV) production and antivirals will be evaluated early in their development giving some confidence in their safety and likely protective efficacy before their use in human subjects.

6. Ability to oversee training programs and advise new manufacturers, national control laboratories, and contract research organizations (CROs) globally in the testing procedures which is imperative to the quality control of new vaccine production and testing of clinical trial materials from new vaccine trials.

Failure to obtain this licence application would compromise the ability to train new staff or retrain existing staff if there is a break in their execution of the test of sufficient duration, leading too loss of skill. With inability to perform the test, in the medium to long term (3-10 years), this would significantly affect the UK's, Europe's and the World's ability to effectively control the release of OPVs. It will also halt the development of new improved vaccine seeds for OPV/IPV production.

How will course attendees use their knowledge or skills in their future careers?

Staff completing this training will then move on to performing work under the main testing licence that covers the intraspinal inoculations required for the WHO Tgm test. This is a very unique skill and requires a high level of dexterity. Being able to perform these procedures means that they can perform quality control testing for polio vaccines and also be involved in testing new vaccine candidates for the polio eradication initiative.

What are the principal learning outcomes from the course?

The objective is to inoculate the dye into the grey matter of the spinal cord. This training stage is considered complete when the inoculator reaches >90% success rate in three consecutive tests of at least 30 animals each.

How are these learning outcomes important to the people on the course?

Progression to the next stage cannot occur until this step is completed.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

1. The World Health Organization uses designated laboratories to provide training in this test globally.
2. European national authorities and medicine regulators rely on this animal model for the OPV safety testing for quality control of vaccines manufactured in Europe.
3. New manufacturers requiring safety testing for new vaccines in development.
4. Clinical trial programmes using this test to test material from the trials. These tests provide data on how successful the trials are and can inform new vaccine development. New novel vaccine strains are being developed for all three polio serotypes, clinical trials for these are currently on-going and they are set to run for a least the next 5 years.

How will you look to maximise the outputs of this work?

An annual workshop with all laboratories performing the test globally is held annually. This is an open forum for all laboratories to discuss issues and achievements in the testing and encourages consistency in the test methodology. This opportunity gives the opportunity to discuss refinements in the test methodology and animal welfare.

Species and numbers of animals expected to be used

- Mice: 4000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice including genetically altered are used. Transgenic mice with the human polio virus receptor are used in the main testing licence. Genetically altered mice surplus from this main testing licence can be used for this project. For training with the ink inoculations mice are not required to be used within a certain age range although only adults are used, both males and females can be utilised depending on availability.

Typically, what will be done to an animal used in your project?

1. Surgical exposure of the injection site. (AC).

2. Intraspinal injection of substances (inert pigment). (AC).

3. Animals will be killed by a Schedule 1 method.

Experience with previously trained inoculators indicates that training speed will vary. Therefore, inoculators will be monitored throughout this important phase of the training, with particular attention in the initial sessions in order to quickly identify trainees who will have difficulty developing the necessary skill: inoculation results will be assessed after each training session. As long as an inoculator is making steady progress towards the target success rate of 90%, training will continue. If progress is poor a full review of the training schedule and results will be initiated, and the trainee may be withdrawn from the programme. Any such review of poor progress will ideally be done early in the programme (typically after about 6 sessions).

As the role of the co-inoculator is simpler, progress is expected to be more rapid though would still normally require on average 3 sessions over 2-3 months, and “loss” of skill less likely. However, progress will still be monitored in the same way as for the inoculator.

What are the expected impacts and/or adverse effects for the animals during your project?

The procedure is non-recovery so any adverse effects would be related to anaesthesia only and minimised by following best practice and veterinary advice, any animals showing any deviation from the norm that is not immediately rectified by adjustment of anaesthesia level will be killed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Expected severity is non-recovery. Only mice are used is this procedure.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 27 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The initial training in identifying the anatomical landmarks can be carried out by looking at pictures and diagrams and watching experienced operators and videos. The initial training in making the skin incision can be obtained on cadavers
The aim is to train the prospective operator(s) to develop the required manual skills to make the inoculation into the correct region of the spinal cord, i.e. into the grey matter. Correct inoculation has been shown to correlate well with two reactions in the test animals: First, a twitch in one or both of the hind limbs as the needle reaches the grey matter of the spinal cord. Second, as the inoculation is made, a distinct tremor/spasm is produced in one or both of the hind-limbs.

Why can't your aim be met by observing or by participating in ongoing research or clinical procedures?

The purpose of this of this project is to train new staff in a procedure in a manual skill which cannot be learnt from just observing. Participation in ongoing procedures is not an option as the operator needs to reach a certain level of skill prior to participation in such procedures.

In a wider context, there are current research and development efforts to replace the WHO Tgm test with molecular assays using next generation sequencing analysis of polio vaccine products. While these methods might take some time to be fully established and validated, replacement of the WHO Tgm test with molecular methods will help reduce the need for this licence in the long-term.

A retrospective assessment of replacement will be due by 27 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

There are a number of assumptions that can be made which aid in setting totals for the 5 year life of the licence:

- Given the nature of changes to currently trained operators, it is reasonable to assume the need to train up to 2 new operators in 5 years (to maintain the current figure of 4 fully trained operators). These will train in both roles. Up to 100 mice are likely to be needed to train for the co-inoculator role. From previous experience, up to 1000 mice will be needed to train for the inoculator role. (2200 mice for 5 years).
- Required annual proficiency testing will require a minimum of 40 mice per trained operator per year. (800 mice for 5 years).
- Each of the 4 trained operators may require up to 2 additional ink sessions annually to retain/regain proficiency. Each session would require a minimum of 40 mice each. (1600 mice for 5 years). With current testing levels this option would probably not be required. However, testing frequencies may change during the life of the licence, making this contingency option necessary
- A conservative estimate for 5 years based on these assumptions suggests 4000 mice will be required (with

a reasonable margin of error factored in).

What in silico or ex vivo techniques will you use during training?

Some aspects of initial training such as identifying the anatomical landmarks and making the skin incision can be carried out by looking at pictures and videos or using cadavers. New methods such as ultrasound guidance have been considered to augment the training process.

Will these techniques reduce animal numbers? If so, how?

The trainers will make sure the trainee is aware of the requirements of the procedures and is as knowledgeable as possible about the requirements prior to performing the procedure. This awareness should reduce the numbers required to be used once performing the manual training themselves.

What other measures will you use to minimise the number of animals you plan to use in your project?

The process is repeated until a satisfactory percentage positive score is reached. This has taken 750 to 1000 mice under the previous training licence. In contrast the training previously carried out required over 1500 mice for each trainee because of irregular intervals in training associated with the need to liaise with another site. Training therefore reduces the total number of animals used.
Having full control of the training process means that potential trainees can be selected based on genuine experience of proficiency in Regulated Procedures and can be monitored closely and constantly by experienced operators to determine genuine rate of progress. Training can be planned carefully to maximise success in a reasonable time frame. Unnecessary repetition of aspects of training can be avoided.
We also use animals surplus from other protocols.

A retrospective assessment of reduction will be due by 27 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mouse model is used including genetically altered, these are the methods used:

1. Surgical exposure of the injection site. (AC).

2. Intraspinal injection of substances (inert pigment). (AC).

3. Animals will be killed by a Schedule 1 method.

This is a non-recovery severity procedure so that opportunities for further refinement are limited to good induction and maintenance of general anaesthesia. It is not necessary for animals to recover from the anaesthesia and they are killed immediately following the inoculation. The vertebral column and spinal cord are then dissected out to allow for precise assessment of the accuracy of inoculation by viewing the distribution pattern of the dye.

Why can’t you use animals that are less sentient?

The main reason for using mice in this training PPL is to prepare for the use of genetically altered mice in the polio studies. Mice are necessary because the main animal model, makes use of genetically modified mice that are susceptible to poliovirus. Our experience, supported through discussion with other organisations performing this test, strongly indicates that it is not possible to observe these required reactions on non-sentient (freshly-killed) animals. In order to elicit the reactions, it is essential that the animals are alive and correctly anaesthetised, but it is not necessary for animals to recover from the anaesthesia - they are killed immediately following the inoculation. The vertebral column and spinal cord are then dissected out to allow assessment of the accuracy of inoculation.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

This is a non-recovery severity procedure so that opportunities for further refinement are limited to good induction and maintenance of general anaesthesia. However, the programme of training and competency is maintained to ensure that the full number of animals under the full protocol is kept to a minimum. In the absence of this training, more animals would be used as tests might fail and need repeating due to suboptimal inoculation. Suffering could also escalate for a test because staff competency would not be as high.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

As this training licence is aimed at preparing for the main licence, we carry out the surgical procedures aseptically and to the same high standards. Therefore, and although there is no post-op care, we follow the general LASA guidance: Guiding Principles for Preparing for and Undertaking Aseptic Surgery. Training protocols were optimised under WHO supervision through experience in different laboratories and discussions in training workshops.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Staff attend conferences on these subjects and are continually researching new methods. The applicant’s expert knowledge in this area and he himself keeping abreast of any new developments. Annual meetings are organised for this forum where presentations are given on the 3Rs and how they have been implemented.
A retrospective assessment of refinement will be due by 27 April 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

204. Translational assessment of cognition in laboratory rodents

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Mouse, Behaviour, Touchscreen, Pharmacology

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The development of effective treatments for devastating mental health conditions such as Alzheimer's disease and schizophrenia is a very high priority. This process however is very complex and associated with a high rate of failure, with drugs found to be effective in laboratory models such as mice often showing no significant benefit

when tested in patients.
An issue that has been highlighted as a potential contributor to this failure rate is the often marked differences between the assessments used to evaluate the effect of a drug on the nervous system in mice (such as swimming in a pool to find the location of a platform) and the analogous assessment used in humans (which might include asking a person to remember a series of words).
The overarching objective of this project is to contribute to efforts to close this so-called 'translational gap' between preclinical research and clinical trials by increasing the similarity between the cognitive assessments used in laboratory mice and humans.
This will be achieved by developing new behavioural assessments for mice that require them to view and respond to images presented on a touchscreen device to earn palatable rewards such as sugar pellets or milkshake. These assessments will be designed to target specific elements of mental health, such as memory, decision making and emotional state. Previous research has shown that it is possible to evaluate some elements of mental health in humans using similar touchscreen tasks and this project intends to expand the number of cognitive features that can be assessed in this way.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This project will generate a number of new behavioural assays for use in laboratory mice that will have much higher similarity to analogous assessments that can be used in humans than are currently available. This will substantially increase the likelihood that a drug that has been shown to improve the function of the nervous system in a mouse will have a similar effect in humans. This will help to reduce the failure rate in clinical trials by potentially eliminating the use of drugs that ultimately will be ineffective in human patients and may also reduce the number of preclinical studies involving animals that are performed to collect the data required to justify entering a new drug into a clinical trial initially.
Expanding the utility of the touchscreen assessment system in mice will also increase the reliability of these experiments as all data is collected automatically by computers and is not reliant on investigators scoring the behavioural performance of mice 'by hand' which can be very challenging to do consistently. The development of 'standard' automated measures of behavioural performance will also make comparison of the touchscreen data collected by different investigators more straightforward than it currently is and eliminate the possibility that inconsistent results are due to differences in assessment method, but rather are due to a genuine biological difference that should be investigated and may reveal important insights.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

This project will involve laboratory mice and over a 5-year period we anticipate that 1500 animals will be involved in this programme of work.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the

likely/expected level of severity? What will happen to the animals at the end?

The focus of this project is the development of non-invasive, low stress behavioural assays in which mice are required to view and decide on a behavioural output in response to simple visual stimuli (such as black and white shapes/patterns) displayed on a touchscreen. These assays will avoid the use of aversive stimuli and situations that mice find stressful such as physical restraint. Therefore, the behavioural procedures which form the basis of this project will be of mild severity.
A proportion of the animals involved in this project will be administered pharmaceutical compounds as part of the process of validating the behavioural tasks. This may require injections which should cause no more than momentary discomfort in the mice. The compounds injected will generally have a wellunderstood mode of action and have previously been administered to mice by other investigators. Therefore, doses will be selected to minimise the potential for non-specific or adverse side effects to occur. Following administration of a compound, mice will be closely monitored for any evidence of adverse effects.
This project may also involve some of the extensive range of genetically altered mouse strains now available. For example, animals that carry alterations in genes known to be associated with particular neurotransmitter systems thought to have a role in supporting the behaviour a particular assay measures will enable confirmation of this association. Similarly, mice altered to carry genetic mutations known to be associated with mental illnesses will determine if an assay designed to measure a particular cognitive characteristic known to be impacted in a particular mental illness is capable of doing so. The mouse strains selected will be those that have already been well-studied by other investigators such that their characteristics and capacity for behaviour will be well-understood. In addition, any strains associated with degenerative conditions (such as Huntington's disease) will only be involved in studies designed to finish well in advance of the age at which the animals are known to develop characteristics with negative health effects, such as movement problems. Such animals will therefore be humanely culled using approved methods well in advance of any potential adverse effects on their health and welfare occurring as a result of their degenerative condition.
To maintain stable levels of behavioural performance, it may be necessary to control the amount of food provided to the mice to motivate them to earn a food reward in the touchscreen tasks. Mild caloric restriction has been shown to have a number of physiological benefits in both humans and animals. Nonetheless, mice experiencing food restriction will be weighed regularly and monitored for signs of ill health. Food restricted animals would be expected to maintain between approximately 85 and 95% of their weight as measured when free fed, and should an animal be found to weigh between 80 and 85% of this value they will immediately be provided additional food and checked to ensure their weight has increased. If an animal is found with a weight below 80% it will be humanely culled using an approved method. At the end of experiments, animals will be humanely culled using approved methods and where necessary the brain and other tissues will be collected for further analysis.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

This project requires access to a complete nervous system with the ability to dynamically select between the full repertoire of cognitive processes and behavioural outputs. It is currently not possible to effectively study complex cognitive processes using *in vitro* methods or computational modelling approaches. Similarly, as the objective of this project is to devise assessments for use in mice, which remain an integral element of the preclinical drug development process, this work cannot be completed in humans.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We are committed to involving the smallest number of animals required to maximise the likelihood of detecting biologically meaningful results in our studies. All experiments will be planned and statistically modelled in advance to ensure the appropriate number of animals is included.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The mouse is the least sentient species capable of supporting the sophisticated behavioural outputs targeted in this programme of work. The mouse and human brain share a number of structural similarities and the mouse has also been shown to be able to perform many of the same cognitive processes present in humans, making it the ideal model system. Equally, a large number of genetically modified mouse strains are available which will enable investigation of the neurobiological mechanisms supporting the behaviour we are studying. A large repository of data concerning the administration of a wide variety of pharmacological compounds to mice also exists which will ensure appropriate doses are used in our studies.
It is anticipated that the majority of procedures performed as part of this project will be of mild severity and cause only momentary discomfort. Animals will be closely monitored throughout for any signs of ill health or distress and humanely culled if necessary.
In addition, studies involving mice genetically altered to exhibit features associated with degenerative diseases (e.g. Huntington's disease) will be completed well in advance of the age at which such animals are known to experience negative health effects (e.g. movement problems), such that these animals will be humanely culled well before their degenerative condition has an impact on their welfare.

Home Office

NON-TECHNICAL SUMMARY

205.Treating a range of pathophysiological retinal insults with gene therapies

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Gene Therapy, AAV, Prevention of vision loss

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this Project Licence (PPL) is to design and test novel gene therapies to treat a variety of eye diseases. Both cell culture systems and rodent disease models will be used to measure gene therapy effectiveness and safety. From the previous PPL, we made several gene therapies to treat glaucoma, a disease that causes permanent visual loss. We will now expand our treatments to other retinal diseases that have an unmet clinical need. These include diabetic macular oedema and both dry and wet-form age related macular degeneration (AMD). Over the tenure of the licence we expect to patent a further four products. The expectation is that one or more of these gene therapies will be progressed into clinical studies.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

In 2010, the global cost of vision care was estimated to be £2.5 trillion for the 733 million people living with low vision and blindness. Eye disorders constitute one of the costliest health considerations worldwide and there remains a high unmet medical need to treat common eye diseases. Current treatments require frequent application, such as daily eye drops to lower eye pressure in glaucoma, or monthly back of the eye injections for AMD. This can cause ocular scarring, in addition to discomfort and inconvenience. Gene therapies involve a single, one-off injection which should protect patient vision many years.

What outputs do you think you will see at the end of this project?

The team are hoping to publish all notable results in high-quality, peer-reviewed journals to inform peers of their successes. Data will also be presented annually at conferences. Key results will also be incorporated into product patents and used in documents for regulatory filing which will support novel products being advanced into clinical trials.

Who or what will benefit from these outputs, and how?

The scientific community will benefit from scientific publications (expectation of 2/year) over the course of the project. The team are also confident of generating at least two novel product concepts within the timeline of the project. It is expected that these products will outperform current market leading therapies for vision loss. Longer term outputs would include clinical trials and approval for patients. Initially, the gene therapies would benefit more affluent countries (due to initial high costs, but also due to the higher proportion of patients suffering from diabetic and age-related eye pathologies). Over time, costs will considerably reduce, and treatments will benefit less affluent countries.

How will you look to maximise the outputs of this work?

We will be publishing data from this PPL in notable peer reviewed journals and presenting results at research conferences. A key output is to patent the constructs so that the treatments can be protected and progressed towards clinical trials so that benefits reach patients.

Species and numbers of animals expected to be used

- Mice: 3000
- Rats: 1100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project uses both adult mice and rats, the majority of which are not genetically altered. The team has extensive experience with both species and the development and testing of gene therapies for the eye. Rodent eyes are anatomically similar to humans, and treatments that are effective in rodents often translate well to human clinical trials. Certain experiments and equipment work better in mice, whilst rat eyes, being larger, allow a more accurate gene therapy dosing regime to be examined.
Young animals would not be suitable for this project, as the retina is less susceptible to damage and the diseases we are investigating are often age related.

Typically, what will be done to an animal used in your project?

All experiments involve injecting a gene therapy into the eyes, usually on one occasion and only whilst the animal is anaesthetised so that it is unaware of the procedure and feels no discomfort. The gene therapies are then given several weeks to reach maximum expression levels before an injury is performed to model a disease. This typically involves the injection of a chemical or protein, a surgical procedure, or the pathology may develop with age in a genetically altered animal. Again, these injuries occur with appropriate anaesthetic and pain relief. During the disease progression various in-life examinations are performed to measure the animal's vision and judge the success of the treatment. These tests are similar to what take place in an eye clinic or at the optician using miniatured rodent equipment. These assessments do not cause any pain but allow extra information to be gathered throughout an experiment.
Animals might also have blood samples taken during the disease progression and after treatment. Blood sampling is more common in the diabetic models to confirm the pathology and ensure sugars in the blood are elevated to a level that can cause visual loss. Sometimes tears or ocular fluids are collected to assess the ocular environment.
At the end of the experiment, animals are killed humanely, and ocular tissues collected and processed.

What are the expected impacts and/or adverse effects for the animals during your project?

Most procedures will not have any adverse effects. The team have performed eye injections and in-life imaging for several years with high success rates and few complications.

Some injuries to the eye can cause temporary swelling and tenderness, which resolves over time. Increased monitoring occurs during, and shortly, after these steps and any animals displaying discomfort or inflammation are treated with appropriate pain relief. Vision in the injured eye should decrease over time to model what happens in human disease. For surgical procedures, the injury is limited to one eye and for genetic strains visual loss occurs slowly over several months. By the time the experiment is terminated, visual loss is expected to be around 50%. For reference, patients often lose 50% of their vision prior to being diagnosed, so this level of vision loss is not expected to impact animal behaviour. Additionally, the gene therapy treatments are expected to preserve sight and reduce any signs of disease within the eye.

The diabetic models will have the greatest impact in relation to adverse effects because the pathology affects the entire animal, not just the eye. Some unavoidable weight loss and possible abnormal behaviour is expected. This is required to match the clinical condition whereby diabetes is a chronic condition and retinal damage occurs gradually over time. I have chosen four different diabetic injury models to mimic different aspects of the disease, allowing me to remove protocols if they are considered less relevant or offer to higher cost to benefit ratio.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

C57BL/6 (a wildtype mouse strain) – mild (50%) moderate (50%)
B6.Cg-Tg(THY-1 YFP) 16Jrs/J (a non-harmful mouse strain that has some fluorescently labelled retinal cells) – mild (60%) moderate (40%) db/db (a genetic diabetic mouse stain) – moderate (100%)

Ins2Akita (a genetic diabetic mouse stain) – moderate (100%)
Wistar, Sprague-Dawley, Lister Hooded (wildtype rat strains) – mild (50%) moderate (50%)

mild = pain or suffering experienced by an animal is low and for less than one day. The animal returns to its normal state within a short period of time.
moderate = animals may experience short-term pain to the eye (i.e. from a surgical procedure), or prolonged impairment to typical behaviour (i.e. a diabetic animal which may become less mobile over time).

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The eye is a complex organ which cannot be accurately examined outside of a living organism. Therefore, animals are required to understand the pathophysiology associated with common eye diseases and ways to prevent blindness.

Which non-animal alternatives did you consider for use in this project?

Where possible, gene therapies are examined in cell culture systems for their ability to transfect and express therapeutic proteins to levels that can influence disease pathways. The use of purified primary retinal cells and retinal organoids (tiny, self-organized three-dimensional tissue cultures) will be investigated over the PPL tenure to limit in-life animal usage.
The group already uses rodent and human retinal explants to study mechanisms relevant to ocular disease. We use these tissues to examine markers and optimise processing protocols and the team have received 3R funding in the past for its non-animal alternative approaches.

Why were they not suitable?

Cell culture systems cannot predict overall efficacy (preventing, attenuating, or stopping pathology). Isolated cells are also not appropriate to examine any immune responses to the gene therapy or evidence of toxicity.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Pilot studies are carried out to calculate appropriate experiment group sizes for full studies. These often involve 3-6 animals to examine reproducibility, safety and to allow further optimisation. Controls are included for all studies and a reference treatment (one which is known to be effective) is often included to benchmark our gene therapies.
For diabetic studies, we have based the estimated number of animals required from scientific literature to obtain statistically and biologically meaningful data. 10-15 animals per group seems appropriate when doing longitudinal experiments due to genetic variability and disease onset.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Statistical advice was sourced from a Biostatistician and experiment study plans are designed to follow appropriate guidelines for animal welfare (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE)) and publication (Animal Research: Reporting of In Vivo Experiments (ARRIVE)). These guidelines help to minimise unnecessary studies, maximise information published and improve reproducibility. Using both eyes of an animal, for non-injury procedures and transgenic animals, also greatly reduces animal numbers and maximises data collection.
Any new surgery, and any new Procedure Individual Licence (PIL) holder will undertake preliminary experiments to generate data relevant to the technique and research question. Similarly, doses will be calculated from effective non-toxic concentrations in tissue culture studies to minimise dose ranges needed to be tested in animals.

To ensure accuracy of data, we also use masked experimentation for almost all studies whereby each animal is given a number and the processing scientist does not know what procedures or treatments each animal has received. In-life results are then not revealed until after the data has been obtained.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To minimise variability, and therefore total number of animals, procedures will be performed on animals of the same strain. Where possible, functional and behavioural tests will be carried out to support anatomical results (e.g. performing retinal vasculature imaging whilst the animal is alive, before supporting these changes with histological retinal sections). The team will also use ocular fluids, bloods and eye tissues to maximise the

amount of data generated per animal.
The team will also provide excess organs and tissues to other researchers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Injury models:

Laser treatment to reduce aqueous outflow - An established model of injury in rats. This model has a high degree of success and is an effective model of glaucoma. Suffering is minimal and complications are alleviated by keeping the eyes lubricated and performing the procedure with pre-defined and optimised laser settings.

Laser treatment to Bruch's membrane - A new technique to the group but one of the most established techniques in the literature to induce damage to the retinal vasculature. Laser spots are directed onto the retina at the back of the eye and the rate at which blood vessels reform and repair are measured. Suffering and distress will be minimal as the injury is self-contained and only applied to one eye.

Microbead intraocular injections – The injection of tiny magnetic beads into the front of the eye (known as the anterior chamber) blocks aqueous drainage channels and causes a gradual rise in IOP. This technique is relatively easy to perform, and eyes and animals remain healthy. There is minimal pain using this model and it allows for post intervention treatment with gene therapies, better reflecting treating of patients with glaucoma.

Crushing of the optic nerve - the optic nerve crush procedure is well established in our team and causes a reproducible level of damage. The optic nerve (which transmits visual information from the eye to the brain) is exposed behind the eye and crushed with forceps for a few seconds. The nerve is then released, the tissues moved back into place and the injury site heals on its own. The technique is relatively challenging, particularly on mice, but the data is essential for assessing neuroprotective gene therapies. Animal group sizes are also small for optic nerve crush studies, due to the consistent level of injury, making it a useful model for testing multiple therapies against one another.

Diabetic animal models:

Ins2Akita mice are a diabetic strain of mouse that lose vision slowly over 6-9 months due to leaky blood vessels in the eye. We will try to use these animals at the earlier end of the disease progression when adverse effects are less likely.

Streptozotocin (STZ) is a toxin that damages cells of the pancreas, reducing insulin levels and therefore resulting in increased blood sugar levels. Similar to the Ins2Akita, blood vessels become leaky in the eye releasing inflammatory cells which can reduce vision. The STZ model induces diabetes as early as 3 days post treatment and can be used in both mice and rats making it favourable when progressing gene therapies to other species.

It is likely only two diabetic models will be required to show efficacy. These will be selected after preliminary testing for suitability and the models that display the least adverse effects or complications will be progressed on the licence.

To avoid adverse effects, all invasive surgical procedures will be carried out according to the Laboratory Animal Science Association (LASA) Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017).

Why can't you use animals that are less sentient?

Due to the complexity of the eye, and as many of the diseases we are aiming to cure occur in adult and aged patients, we need to use appropriate models. We require the use of mammals due to the similarity in the visual system and the length of time for a treatment to express or a disease to progress means procedures under terminal anaesthesia are not applicable. The injury models and gene therapies being designed are relevant to human disease and therefore have a high likelihood of transitioning to patients.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We've introduced a scoring system (1-10) for all procedure with anything lower than 7 having additional checks post-surgery. These animals will be examined thoroughly throughout the study for evidence of ocular damage and given post-operative antibiotics if required. We will also monitor the weight and behaviour of diabetic animals throughout studies to better understand the pathology and to intervene as early as possible. Each researcher will keep hold of these records and use them to improve their experiments. From experience, researcher scores consistently increase from 5's and 6's to 8's and 9's when performing surgeries on a regular basis.
A health assessment score sheet has also been created to identify complications linked to surgery or diabetic strain to help researchers and animal technicians agree on a correct course of action based on a multitude of scores and criteria.

The group will also have a picture monitoring catalogue detailing what to expect post-surgery and possible adverse expects. The team also train routinely on cadavers which allows them to perfect their surgeries and techniques before beginning pilot studies. This reduces variability whilst helping reduce animal usage through failed procedures.
A shared spreadsheet will be maintained within the group which details the course of all animals through all procedures performed. This spreadsheet will record each animal, every procedure performed on that animal, the actual severity reached, additional comments to the study and how the tissues were used and stored. This system has worked very well in the past.
Over the tenure of the licence, we will also employ an experienced animal technician to oversee procedures and work closely with the animal facility technicians. Maintaining active communication with the facility animal technicians is vital and will allow us to immediately identify problems. In the past, I have also given presentations to the animal facility staff and managers about the nature of our experiments and studies. This helps the facility staff understand what we are doing and why certain adverse effects can arise and why others are not expected.
Animals will also be housed in groups to enable social interaction and grooming. From experience, rarely do our

animals fight after surgery and open wounds are not expected. We have experienced minimal signs of stress or discomfort from similar procedures in the past and expect a good quality of life for the animals on this licence.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Publications will follow the ARRIVE and PREPARE guidelines using the 'Animal Research: Reporting In Vivo Experiments' template. LASA publications will also be referred to in addition to any other best practice guidance articles on surgery and procedures.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will be applying for 3R funding throughout the tenure of the project which requires constantly keeping up to date with 3R advancements. We also seek to collaborate on several 3R projects allowing us to learn and implement new ways to replace, refine and reduce animal usage.
Information will also be periodically checked from www.ubs.admin.cam.ac.uk/3rs/3rs-search-tool, www.animalcare.ubc.ca/animal-care-committee/sops-policies-and-guidelines and www.arvo.org/About/policies/statement-for-the-use-of-animals-in-ophthalmic-and-vision-research/ that provides detailed guidelines for working with animals for eye related research.

Home Office

NON-TECHNICAL SUMMARY

206. Treatment of abnormal retinal development

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to demonstrate, using albinism as an example, how to develop precision medicine pathways to prevent any child who has inherited a retinal form of blindness from losing their sight.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Inherited eye disease is one of the most common causes of childhood blindness, affecting circa 420,000 children worldwide. Oculocutaneous albinism (OCA) is an example of an inherited eye disease that illustrates why the control of blindness in children is a high priority within the World Health Organisation's VISION 2020 — The Right to Sight programme. Albinism affects up to 1 in 1000 people and is characterised by pigment deficiency of the skin, hair and/or eyes, abnormalities of retinal development and visual impairment. The average best-corrected visual acuity (VA) in OCA is below the UK eyesight standard for driving. Moreover, there are significant effects on school performance, employment and quality of life and most patients are registered as sight impaired in the UK. Despite significant advances in understanding the molecular basis of inherited eye diseases e.g. albinism, there remains a paucity of effective treatment options. This project aims to remedy this deficit.

What outputs do you think you will see at the end of this project?

By the end of the project we hope to have established:

1. The optimal L-DOPA (Levodopa) dosage parameters that can safely obtain maximum rescue of visual function in oculocutaneous albinism (OCA), in preparation for a future randomised controlled trial (within the next 5 to 10 years).

2. Provide new insights into the mechanisms underlying abnormal retinal development in albinism, potentially identifying novel pathways to interrogate in future work.

3. Development of additional novel therapies for albinism and other inherited retinal diseases. This will lead to phase 1/2 randomised controlled trials (within the next 10 years) and establish proof of concept for developing effective precision therapies for other inherited retinal diseases.

In addition, the adoption of a longitudinal study design using non-invasive investigative techniques such as electroretinograms (ERG), optical coherence tomography (OCT) and OptoMotry™ to demonstrate improvements in retinal development & visual function in response to an intervention in a murine model has not been done previously. If this is successful, it may serve as a model for other vision scientists working on drug development studies and has the added advantage of reducing the numbers of animals needed to demonstrate a significant result.

The information from this project, including the study protocols, will be made freely available via publication in peer-reviewed journals, in order to benefit other researchers (e.g. paediatric ophthalmologists and physicians) who are involved in the development and assessment of novel therapeutics which target abnormal retinal development in early childhood.

Who or what will benefit from these outputs, and how?

By the end of the project we hope to have established:
The optimal L-DOPA (Levodopa) dosage parameters that can safely obtain maximum rescue of visual function in OCA, in preparation for a future randomised controlled trial (within the next 5 to 10 years).
Provide new insights into the mechanisms underlying abnormal retinal development in albinism, potentially identifying novel pathways to interrogate in future work.
Developed additional potential treatments for albinism and other inherited retinal diseases. This will lead to phase 1/2 randomised controlled trials (within the next 10 years) and establish proof of concept for developing effective precision therapies for other inherited retinal diseases.
In addition, the adoption of a longitudinal study design using non-invasive investigative techniques such as electroretinograms (EGG), optical coherence tomography (OCT) and OptoMotry™ to demonstrate improvements in retinal development & visual function in response to an intervention in a murine model has not been done previously. If this is successful, it may serve as a model for other vision scientists working on drug development studies and has the added advantage of reducing the numbers of animals needed to demonstrate a significant result.
The information from this project, including the study protocols, will be made freely available via publication in peer-reviewed journals, in order to benefit other researchers (e.g. paediatric ophthalmologists and physicians) who are involved in the development and assessment of novel therapeutics which target abnormal retinal development in early childhood.

How will you look to maximise the outputs of this work?

Public Engagement & Education

Our approach towards developing an effective treatment for albinism serves as an excellent example of how clinical observations at bedside can be taken back into the laboratory for more detailed mechanistic investigations and novel therapeutics development which can then be translated back into clinical practice. This is a good story which we will disseminate to the public through outreach activities, press releases etc. Those affected by the condition (sufferers and parents) will be especially interested, and we will make efforts to reach this audience. We will communicate the progress of our study to the patient community on a regular basis in conjunction with our patient and public involvement (PPI) study group. The results will be presented to the patients by liaising with patient focus groups, attendance at open days, where patients, parents and carers will be informed of the progress of the study. The results will be publicised in patient newsletters and other resources. We will also utilise social media resources such as Facebook and Twitter feeds to disseminate up-to-date information about the study.
Communication of findings to the scientific community will be disseminated by presentations at national and international conferences in addition to publications in peer reviewed high impact ophthalmological and medical journals. In addition to publication, the results will also be communicated on relevant server lists. Progress of the study and results will be regularly disseminated at ongoing teaching events and public lectures.

Species and numbers of animals expected to be used

- Mice: (1) Normal pigmented control mice: 130, (2) Mice with ocular cutaneous albinism (OCA) type 1: 430, (3) Mice with the ocular albinism (OA) subtype: 230, (4) Reporter Mice (i.e. mice who have been genetically modified so that specific cell-types in the retina are labelled, for the purposes of monitoring the dynamic processes that take place during retinal development): 100.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of

the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We have demonstrated that eye development and vision in oculocutaneous albinism (OCA) can be improved through L-DOPA (Levodopa) supplementation, if administered during the critical period of neuroplasticity (i.e. younger than 18 months of age). Unfortunately, L-DOPA (Levodopa) is unlikely to be effective in ocular albinism (OA), as it normally acts through the OA1 receptor to regulate eye development. This receptor does not work correctly in OA.
Targeted treatment(s) are needed that are not age-dependent, or dependent on an intact OA1 receptor. Pigment epithelial derived growth factor (PEDF) treatment is of interest, as PEDF acts after the OA1 receptor to regulate eye development. Another option is to expand the critical treatment period into adulthood, using ciliary neurotrophic factor (CNTF) for which proof of concept has been demonstrated in other retinal conditions.

Before these and other potential treatments can be developed and translated into clinical practice, we need to prove that they can safely improve eye development and eyesight in infant (L-DOPA
(Levodopa) & PEDF) and adult (CNTF) mice with human albinism. We will also need to compare each of these treatments, under the same conditions so that we can identify which one is the best treatment.

Typically, what will be done to an animal used in your project?

A typical mouse will receive a treatment, which may be dissolved in their drinking water, given as an eye drop or a single injection into the eye (like the injections given to humans to treat age-related macular degeneration). This mouse's eye development and eyesight then needs to be checked and followed over time. The eye development can be checked using a non-invasive retinal scanner called an optical coherence tomography (OCT). The mouse optical coherence tomography (OCT) is an adapted version of the human optical coherence tomography (OCT), which allows us to take highly detailed images of the retina. In order to measure eyesight, we will use electroretinography (ERG), which measures the electrical activity being generated by the retina in response to light, a bit like how an EEG measures brain activity. The mouse will have a maximum of six optical coherence tomography
(OCT) and electroretinography (ERG) examinations at: 4 weeks, 5 weeks, 6 weeks, 2 months, 3 months & 4 months of age, so that we can monitor how their retina and eyesight develops over time. These examinations will be carried out, after we have put the mouse to sleep using a small injection of anaesthetic. This is to minimise any possible distress of discomfort that the mouse may experience.
Like infants who like to follow stripes and patterns, mice also have the same reflex. We can use this reflex to check how well the mouse is seeing when they are awake. In order to do this, the mouse stands on a central platform. Black and white stripes of varying thicknesses are rotated around the mouse. If they see the stripes, they move their heads to follow it. The finer the stripe that the mouse follows, the better their eyesight. We will do this test twice at 2 and 4 months of age.
Mice may experience some side effects from the treatment that they are getting. This can include changes in their normal behaviour, anxiety, memory problems and muscle weakness and coordination problems. If mice are suspected have having these side effects, they will undergo some additional assessments at 7, 11 and 15 weeks of age. This will include observing their normal behaviour in their home cages (home cage activity), examining their normal tendency to explore by placing a novel object into their environment (novel object exploration test) and assessing their anxiety levels by observing their tendency to avoid open spaces (open field arena) or avoid falling off of an elevated platform (elevated plus maze). Muscle strength will be measuring the time it takes a mouse to fall of an inverted square of wire mesh held 40-50 cm over a padded surface (Kondziela's inverted screen test). Coordination will be testing by recording how long a mouse can stay on a rotating rod, rather than falling onto a padded platform 30-50 cm below (Rotarod test). Memory testing will only be conducted once at 15 to 16 weeks of age and will involve either the novel object exploration test or the radial maze test. With the novel object exploration test, the mouse will first have several sessions over two day to explore and become familiar with a novel object. Following this a second novel object is introduced, and

provided the mouse has remembered the first object, they should spend more time exploring the second object. The radial maze test involves training the mice for 4 days to locate food pellets in baited arms of the maze. Following this, they will undergo several trials over a number of days, in which the maze will have specific arms that are baited, that are fixed for each trial. Their ability to remember which arms are baited can then be assessed based on the number of arm entries required to locate to acquire all of the food pellets.

What are the expected impacts and/or adverse effects for the animals during your project?

The individual procedures planned in this project are expected to cause no more than mild transient pain or distress and no lasting harm to the majority of the animals.
It is possible that some mice may experience side effects from their medication, which can manifest as loss of appetite and weight, dehydration, lethargy or hyperactivity, twitching, trembling, agitation, sleep disturbances. This is expected to be a rare occurrence and to date, we have observed no side effects in the animals that we have treated with human-equivalent doses of L-DOPA (Levodopa). In the rare event that this occurs, affected mice will quickly receive routine medical treatment appropriate to the presenting symptoms. Any animal that is in chronic pain or distress, which cannot be relieved, will be immediately euthanised.
Behavioural and neurological tests may cause temporary (a few minutes) distress to animals, however, to limit/avoid such distress, mice will be allowed to adapt to the environment prior to testing.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severity for 90% of the animals in this project is expected to be moderate, as each treated mouse will have up to six separate examinations under anaesthetic throughout their lifetime. The remaining 10% (i.e. animals not undergoing several repeated examinations over time) are expected to be mild or sub threshold.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animal use in this project is necessary for two specific reasons:

1. In order to establish L-DOPA (Levodopa), PEDF, CNTF as valid treatment options for infants, young children and adults with albinism it is necessary to identify with as much precision as possible the therapeutic timing window and optimal dosages in a genetically well characterised model at specific time-points. In humans, albinism is a genetically mixed group of conditions, making it extremely difficult to diagnose the specific genetic subtype at an early age, and limiting the precision with which the potential therapeutic window can be determined. In addition, proof of concept for each of these potential treatments needs to be demonstrated prior to proceeding to human clinical trials.

2. To provide post-mortem ocular tissue for investigation of the molecular and cellular mechanisms of L-DOPA (Levodopa) mediated rescue of retinal development and function in albinism, and to determine potential novel targets for future treatment studies.

Which non-animal alternatives did you consider for use in this project?

Ex-vivo tissue and cell
line cultures.
Why were they not suitable?

Demonstration of improvements in both retinal morphology and visual function in albinism in response to potential treatments cannot be achieved using in-vitro cell culture & expression techniques or protein functional studies in ex-vivo tissue and cell line cultures.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

In order to determine the appropriate sample size & minimise the numbers of animals needed for this project; a power calculation was performed based on previously reported electroretinogram data in this murine model. In comparison to controls, a sample size of n=10 in each group will identify a difference in a-wave and b-wave amplitudes of approximately 24% and 26%, respectively (where alpha=0.05 and power=90%).
Histology: Retinas stained with H&E will be used to count nuclei. Previous studies which focused on cell counts of the outer nuclear layer nuclei used an n=4 retinas per group..
Transmission Electron Microscopy (TEM): Previous studies from the literature suggest that the optimum number for TEM studies of retinal synapses require analysis of a minimum of 10 slices, from a minimum of 3 mice per group. However, because these experiments cannot be performed blinded, we will be using a slightly increased number (n=5) to ensure unbiased results.

Immunofluorescence and Proximity Ligand Assay: These are qualitative studies, where there is no standard deviation. Eyes will be collected from the same mice used for other studies, as TEM, histology or protein/genetic studies, therefore, there will be a minimum n=4 eyes/group that will be available for confocal imaging.

Cell sorting: Previous studies sorting retinal cell types suggested that 4 mice retinas yielded 1.5– 2.5×106 rod photoreceptors in a volume varying between 4-10 ml. Considering that mammalian cells normally have a content of RNA around 10-30pg, 4 mouse retinas will have between 15 and 75µg of RNA. Depending on the experiment where cells will be sorted, the sample size will vary.
Transcriptomics: Samples will be run by RNAseq; paired end 2x75bp at 30 million reads (on average per sample). Typically, retinal transcriptomics are run with an n=3 of samples that will, depending on the system, come from one mouse or a pool of retinas. The minimum amount of RNA required is 1µg. Taking into consideration the calculations above, retinas can be sorted individually to prepare transcriptomics samples, therefore needing 3 mice/group.

Protein studies (WB/ELISA): As it has been previously described for retinal development studies of protein by western blot, I will use n=3 retinas per homogenate and 3 different homogenates per group.
Genetic studies (PCR): Previous studies have indicated that it is necessary to have 3 biological replicates to perform statistical analyses. As we do not know a priori the genes that will be analysed (dependent on the transcriptomics results), I will plan an n=5 mice/group to ensure unbiased results for different depths of the candidate genes.

OCT: In our previous study (unpublished own data), the response within each subject group was normally distributed with standard deviation of 3. If the true difference in the experimental and control is 5, we will need to study 7 experimental subjects and 7 control subjects (alpha=0.05 and power=80%).
OptoMotry™: We have used data from preliminary experiments to obtain an estimate of the standard deviation of 0.55. Based on this, a sample size of 5 mice/group will detect a difference of at least 25% between groups (alpha=0.05 and power=80%).
In all cases, we have also included a margin of 10% in the numbers provided and rounded the figures up to the nearest 10.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The design of individual experiments will be optimised to ensure that the maximum amount of data is obtained from the minimum amount of resources. A randomised block design will be used to determine treatment allocation and a mixed cross-sectional & longitudinal study design has been planned which will minimise the number of animals needed for this project.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The work in this protocol can be maximised by interrogating the underlying cellular and molecular mechanisms responsible for the recovery of retinal function, in the male OCA mice. These mice are currently not being used in this study, for the longitudinal in-vivo assessments of retinal structure and function in response to potential treatments. The underlying cellular and molecular mechanisms responsible for L-DOPA (Levodopa) mediated recovery of retinal function in albinism are unclear. Understanding the molecular and cellular mechanisms of Levodopa mediated rescue of visual function in Albinism is the key to developing novel therapeutics for the visual problems associated with albinism. Therefore, a subset of male mice will be culled at different ages/time points throughout the treatment protocol for the purposes of interrogating the molecular and cellular effects of L-DOPA (Levodopa), PEDF and other potential treatments on ocular and cortical development and morphology in albinism, thus ensuring maximum output from all available tissue.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

For this study, we have chosen the only genetically well characterised animal model of albinism in which preliminary data exists for the use of oral L-DOPA (Levodopa) (including drug dosages and formulation) in safely rescuing retinal function – the C57BL/6J-c2J murine model of OCA1. This making them an ideal model for longitudinal monitoring of in vivo retinal development response to different therapeutic candidates.
In order to determine treatment efficacy, we plan to use longitudinal electroretinography (ERG), optical coherence tomography (optical coherence tomography (OCT)) and OptoMotry™ assessments of retinal, development, structure and function. Baseline electroretinogram values have been established as a measure of visual function in both control and albino mice. Optical coherence tomography (OCT) imaging of the mouse retina has been used successfully to identify morphological changes in degenerative retinal diseases and has

been shown to be comparable to histological quantification. Electroretinography (ERG) and optical coherence tomography (OCT) are both non-invasive and well established examination techniques, that can be carried out sequentially under the same general anaesthetic. This minimises any potential pain or distress experienced by the mice. OptoMotry™ visual function examinations is also a well-established technique, where we observe the mouse's response to a visual stimulus consisting of rotating set of black and white stripes. This is carried out in awake mice, is non-invasive and may cause temporary (a few minutes) distress to animals until they become accustomed to the test environment. This results in less distress than traditional Morris water maze visual assessments, which carries a risk of drowning and hypothermia.
In order to assess neurodevelopment, we have chosen to use well established non-invasive tests that can be conducted in awake mice, including observation of home-cage activity, novel object exploration (exploratory behaviour) open field activity and elevated plus maze (anxiety related behaviour). For muscle strength (Kondziela's inverted screen test) and coordination (rotarod) we have also selected two well-established, non-invasive and short tests that can be performed in awake animals, Apart from observation of home-cage activity (60 minutes), all of these tests can be conducted in 5 minutes or less, thus minimising any potential distress or discomfort experienced by the animals, and facilitating longitudinal assessments. For memory testing we have selected the novel objective exploration test or the radial arm test as the best options for assessing memory, as these have been validated, with established protocols and will result in less distress than Morris water maze memory assessments, which carries a risk of drowning and hypothermia.

Why can't you use animals that are less sentient?

In order to determine treatment efficacy, we need to perform longitudinal electroretinography (ERG), optical coherence tomography (OCT) and OptoMotry™ assessments of retinal, development, structure and function. These tests can only be performed in animals to whom we can:

1. Administer oral, topical or systemic medications

2. Perform longitudinal electroretinography (ERG), optical coherence tomography (OCT) and OptoMotry™ assessments

The tests have been specifically designed and optimised for mice and requires animals that have reached a sufficient stage of maturity and sentience for the tests to be performed safely and reliably.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In order to minimise the numbers of mice needed & any possible distress, the functional & anatomical effects of administering L-DOPA (Levodopa) to mice at different ages on retinal development will be determined by performing longitudinal, non-invasive electroretinography (ERG) & optical coherence tomography (OCT) examinations under general anaesthesia. Animal suffering will be minimised in accordance with ASPA guidance. All animals undergoing any form of examination will be studied for a maximum of 30 minutes per session and will be given appropriate rest periods between examinations. Local (such as lignocaine) and general anaesthetic (including ketamine and isoflurane) will be administered for surgical procedures followed by systemic analgesia. Mice undergoing surgery will be given an appropriate rest period before further study, typically one week according, or when animal resumes normal behaviour (eating and drinking) according to established protocols.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will conduct all our experiments in accordance with the most recent guidance from the NC3Rs and the ARRIVE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Throughout the project, we plan to stay informed about advances in the 3R's using a combination of the resources at the available at the NC3R's website: https://www.nc3rs.org.uk/3rs-resources and the NC3Rs monthly newsletter.
In order to implement these advances effectively, we plan to:

1. Disseminate them to all members of the team
2. Incorporate them into our training
3. Embed them where possible into our local experimental guidelines and protocols.

NON-TECHNICAL SUMMARY

207. Tuberculosis infection and treatment model for active tuberculosis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Tuberculosis, Mouse model, Therapy

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to test drugs or drug regimens using Cornell tuberculosis treatment model to examine their activities against the TB causing bacterium, *Mycobacterium tuberculosis* in actively growing and persistent forms which co-exist in the organs of animals with active TB infections.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Tuberculosis caused by the bacterium called *Mycobacterium tuberculosis* kills nearly 1.8 million people every year worldwide. One of the important characteristics of the disease is that the bacterium has an unusual ability to survive for extended periods of time in human body despite human immunity and anti-TB drug treatment.
It has been estimated that about 2 billion people, equal to one-third of the world's total population, are infected with the bacterium in whom it causes unnoticeable latent infections that lead to a 5-10% lifetime risk of active disease. Current tuberculosis treatment needs at least 6 months with four drugs to cure the patients. This long-term treatment is extremely difficult to implement especially in developing countries. Poor patient compliance will lead to treatment failure, high disease relapse and promote anti-TB drug resistance. We desperately need new drug regimens which can shorten the treatment duration and prevent TB relapse.

What outputs do you think you will see at the end of this project?

The novel drug regimens tested in this study will provide novel information on their bactericidal and sterilising activities against active TB for the clinical studies in patients
The research has its novelty which will be disseminated to the wilder scientific community by high impact publications and in national and international conferences.

Who or what will benefit from these outputs, and how?

Patients will directly benefit from the research project as novel drug therapy will shorten treatment duration and prevent disease relapse. The activities of the drugs against *M. tuberculosis* have been shown in test tube experiments, therefore the therapeutic efficacy to kill the bugs in animals will be observed at early stage of the project which contribute or predict further impact on disease treatment.
The findings of the projects will be rapidly published in high impact journals which introduce new field, novel treatment strategy, novel therapeutic drugs to advance academic understanding and research in the infection and treatment fields.
This information will advance academic knowledge and UK competitiveness. In the public sector, the work will most directly impact on the academic research community and healthcare professionals who care for patients with infectious diseases such as tuberculosis. Findings in the project will also benefit veterinary professionals and the treatment of the disease in animals and livestock.

How will you look to maximise the outputs of this work?

The findings in this project will be published in high impact journals and disseminated via national and international conferences. The scientific and commercial communities will be made aware of all positive and negative results or approaches to avoid unnecessary duplication of the findings using animals.
The results produced in this project will enable us to collaborate with academic research sectors and establish international collaborative networks that exchange ideas, skills, and jointly produce publications which will reinforce the dissemination of new knowledge produced in the project.
This will also enable us to collaborate with clinicians in the hospital and clinical settings to fully understand the pharmacokinetics and pharmacodynamics of the drug regimens and the management of TB patients with the new drug regimens.
The research outcomes from this project will enable us to apply for further grant supports to advance the findings of the project.

Species and numbers of animals expected to be used

- Mice: 2500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are chosen because most of the standard refined infection and treatment models are using mice and most of the knowledge, experiences and scientific data come from mouse infectious models. Mice are the closest compatible animals to predict and reproduce the effects of antimicrobials potentially applicable for human usage. The infection models we proposed are well characterized by infection dose, route and disease progression. Adult mice will be used as they have matured immunity against bacterial infection. Also, adult mice have fully developed organs such as liver that is an important organ for drug metabolism.

Typically, what will be done to an animal used in your project?

Mice will be infected with TB bacteria via injection or inhalation to induce diseases in their organs such as lungs or spleens. The diseases progress normally takes 2 to 10 weeks. Treatment with anti-TB drugs will be given to the animals orally or by injection (a similar way to those are given to patients) at 2 to 10 weeks after M. tuberculosis infection to reduce the bacterial numbers in the infected organs. The treatment will be 5 days per week for up to 20 weeks. At the end of the treatment, the remaining mice will be given corticosteroids for 8 weeks to check disease relapse as a result of the treatment outcome. The experiment will be terminated. The duration from infection of the animals to completion of the experiment is within 44.5 weeks. Blood sampling to check the drug serum levels will be performed via superficial veins or cardiac puncture which will only ever be conducted under terminal anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

Mice normally do not show clinical symptoms if we infected mice with a low or medium dose of the bacteria. We will control the inoculation size and duration of infection before treatment is started which only allow bacteria to grow in the lungs or other organs of mice but do not induce clinical signs. The medicines used to treat TB will not cause side effects to harm the animals. Side-effects of corticosteroids in this model are immune-suppression which can result in clinical infection from other bacteria. We will give the animals antibiotics to prevent other bacterial infections. The steroid also induces reactivation of dormant TB which may transfer to active TB. The animals will be closely observed, if animals show any of the adverse effects, they will be humanly killed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected maximal severity will be moderate which will be monitored and controlled using scoring system. The intervention procedures given to the mice individually are mild, for example, IV, IP injection or oral dosing. However, repeated oral gavage may have a cumulative effect to cause discomfort. In this model, approximately 80% of the time, mice are treated with anti-TB drugs or steroid via oral dosing.
The low and intermediate doses of bacterial infection to the mice are mild. However, at the follow-up period when steroid is given and no anti-TB drugs are administrated to the mice, mice may develop active infections due to disease relapse, one-third of mice at the end of the experiment may experience moderate severity

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

One of the most important steps in preclinical studies of anti-TB drug discovery and development is to test the activities of drugs or drug regimens using animal models. Nearly all the antimicrobial agents have been tested using animal models before studied in human trials. The activities of the anti-TB drugs against TB causing bacteria have been tested in our test tube experiments and their toxicity has been examined using animal or human cell cultures. However, the environment in a live animal is much more complex than that in a test tube or a cell culture. This means that it is necessary and important to study the bacterial colonization and the drug activities in living animals to assess the effectiveness of the treatments. Therefore, whole animals are needed.

Which non-animal alternatives did you consider for use in this project?

The activities of the antibiotics or drugs against TB causing bacteria have been tested in our test tube experiments and their toxicity has been examined using animal or human cell cultures. The anti-TB drugs have been used clinically in patients.
Cytotoxicity assays using mammalian cell lines, Ames test for mutagenesis, comparative in vitro metabolism with human, rat, minipig hepatocytes and the HerG test have been considered as alternatives.
Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Minimum Stationarycidal concentration, Minimum Dormicidal concentration have been considered as measures of efficacy of antimicrobial. Drug solubility, stability, tissue penetration, metabolism, excretion, formulation can be tested in vitro.

Human samples such as sputum can be used as part of alternative to animals.

Why were they not suitable?

These in vitro tests have limitations in predictive power when compared to the whole animal. There are factors in the whole animal which cannot be mimicked in vitro. For example, simple cytotoxicity assays have been used with partial success to predict animal and human toxicity and to estimate starting concentrations for animal toxicity studies. But the pathways leading to drug toxicity are very different between cell lines and whole animals. Cell-based assays typically lack metabolic competence and often miss chemicals that require bio-activation in a whole animal. They also fail to provide data on some of the most important toxic mechanisms, for example those involve organ- or cell-type specific physiology.
Although Minimum Inhibitory Concentration of an anti-TB drug in particular, is used to predict drug efficacy in humans, the pharmacokinetics and pharmacodynamics are very different in a whole animal. Also, bacterial colonization and growth in human organs and tissues are very different from those in the test tubes, therefore results obtained from in vitro studies cannot often directly predict biological responses of organisms to drug exposure in the animal. Therefore, whole animals are needed.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

In order to evaluate the efficacy of the TB drug regimens, bacterial numbers in the infected organs will be

examined at different intervals for 14 to 20 weeks after treatment is started. The bacterial numbers will be monitored as colony-forming units on agar plates and most probable numbers in the liquid medium. For each treatment group, these bacterial numbers will normally be taken from the infected organs from 4 animals at early time points and 6 to 8 animals at the late time points. After completion of the treatment, about 20 animals will be kept for 8 weeks to examine TB relapse. These numbers of animals used in each time point are derived from power calculations which optimize the numbers of animals required to obtain the maximal study outcome with minimal numbers of animals. Based on data from 22 previous treatment arms performed in the TB model, a range of linear elimination rates from -0.16 to -1.07 log CFU/wk have been observed with an average standard deviation of 0.23. With a sample size of 60 animals per treatment arm and an alpha of 0.05, a difference in elimination rate of 0.19 will be detected with greater than 90% power. For the TB model, we will use 500 mice/year with about 7 treatment arms and one control. For the 5 years, the maximal of 2500 mice will be used.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Experimental designs and methods of analysis of the results have been discussed with professional statisticians and with those who have experience in the field. The design of individual experiments will usually involve factorial designs, which maximize the information obtained from the minimum numbers of animals.

We will also use the NC3R's Experimental Design Assistant online tool to design our experiments to ensure we will use the minimal numbers of mice to produce significant scientific results.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will select the effective drugs with no toxicity using well-defined in vitro tests before applying them to animals to reduce the number of animals used. For any new drugs being tested in the infection models, pilot studies will be carried out with small numbers of animals to assess the activities of the new drugs.
We will employ computer modelling using the data derived from our previous studies to evaluate the drug efficacy using the numbers of the animal used in the studies. Model-based clinical development of antimicrobial and drug combinations has been used in our previous studies. We will continue to employ those methods to optimise the number of animals used in the study to provide us with the maximal scientific outcome.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Tuberculosis infection and treatment model will be used in this project to test anti-TB drug activities against *Mycobacterium tuberculosis* that causes tuberculosis.
The TB mouse model was established more than 60 years ago. The current TB drugs used to treat human TB were firstly tested using this model. During these years, the model has been refined in different research groups around the world which produced the optimal scientific outcome with the least adverse effects to the animals. In this model, mice are infected with the bacterium that causes TB, the immune response of the animals against TB will be established at 2 or 3 weeks after infection, that can inhibit TB bacterial growth but will not eliminate the bacterium. The infected mice will be treated with TB drugs, that will reduce the bacterial numbers in the infected organs and control the infection.

Why can’t you use animals that are less sentient?

Mice are chosen because most of the standard refined infection and treatment models are using mice and most of the knowledge, experiences and scientific data come from mouse infectious models. Mice are the closest compatible animals to predict and reproduce effects of antimicrobial potentially applicable for human usage. The infection and treatment model we propose is well characterized with infection dose, route, disease progression and treatment management. Also, mice are less sentient to tuberculosis showing a strong immunity against TB bacteria.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To reduce adverse effects, animals will be closely monitored throughout the experiments and will be provided with environmental enrichment such as fluid and warming pad to reduce discomfort. It is proposed to utilise optimal experimental techniques with minimal intervention performed by well-trained persons. Infections will be rapidly controlled by providing treatment to the animals with anti-TB drug therapy. The experiments will be terminated at the earliest possible stage when a satisfactory scientific outcome is produced. All these measures will reduce distress and suffering to the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The ARRIVE guidelines
Guidance on the operation of the Animals (Scientific Procedures) Act 1986 (Home Office)
The Harm–Benefit Analysis Process (Home Office)
LASA Good Practice Guidelines (Administration of Substances and Collection of Blood Samples) will be followed for dosing and blood collection

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Receiving newsletters, information, and guidance on the latest knowledge to improve laboratory animal welfare with the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs). Also, to use NC3Rs experimental design to ensure our methods and findings are robust and reproducible.
Having regular meetings and discussions with the local animal Establishment and close liaison with the Named Information Officer.
Attending the internal and external training courses available to learn and obtain updated information about the advances of the 3Rs in animal research.

Home Office

NON-TECHNICAL SUMMARY

208. Uncovering Novel Targets for Pulmonary Arterial Hypertension

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Pulmonary hypertension, Therapy, Pharmacology, G protein-coupled receptors

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of our research is to advance the understanding of pulmonary arterial hypertension and uncover new drug targets for the disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There is currently no cure for pulmonary arterial hypertension and current treatments, such as phosphodiesterase 5 inhibitors, calcium channel blockers, prostacyclin receptor agonists and endothelin receptor antagonists, which have little effect on disease prognosis, can cost over
£300,000/year/patient. The only way to completely reverse pulmonary arterial hypertension is a lung or heart/lung transplant. Understanding the triggers of the disease and uncovering new drugs for pulmonary arterial hypertension is essential.

What outputs do you think you will see at the end of this project?

We have identified novel proteins, such as G protein-coupled receptors, which are significantly increased in the blood vessels of patients with pulmonary arterial hypertension compared to patients with normal pulmonary pressure. G protein-coupled receptors and their downstream pathways are excellent drug targets: >30% of current approved drugs target GPCRs, a number of which are used to treat pulmonary arterial hypertension. The research, although basic science in nature, is aimed at providing a greater understanding of pulmonary arterial hypertension and validating novel drug targets for the disease that have not been previously been described. The results generated from the studies will have wide implications for the field of research in pulmonary arterial hypertension and be published in peer reviewed high impact journals, presented at national/international conferences and by engagement with the public and patient groups. Furthermore, increasing the understanding of PAH could lead to additional understanding of other lung pathologies such as COPD, HIV infection, connective tissue disease and congenital heart disease.

Who or what will benefit from these outputs, and how?

In the short-term, researchers (both in nationally and internationally) will be able to incorporate this new target into current models of pulmonary arterial hypertension. It is envisaged that validating novel G protein-coupled receptor targets will lead to interaction with major drug companies to develop screens for drugs or humanized antibodies directed to these proteins that could inhibit its activity, which if successful will generate valuable tools for clinical research. The insights into disease pathogenesis will also help the medical community understand the disease process. It is hoped that the main beneficiary one-day, in approximately 5-10 years, from this work will be patients with pulmonary arterial hypertension, who currently have no curative treatment available.

Once potential therapeutic agents are identified it is anticipated that they will be tested in-vivo. Such invivo experiments will provide evidence for the clinical utility of these agents for pulmonary arterial hypertension and intends to lead to novel therapies for the disease, although full preclinical testing may be required. Since existing drugs target GPCRs and phosphodiesterase, depending on the targets we uncover preclinical testing would already have been carried out, therefore drugs could fast track to the clinic.
Fast track to clinic is important as although pulmonary arterial hypertension (PAH) is relatively rare (approximately 3000 cases in the UK), it has a poor prognosis and current treatments costs up to £300,000/year/ patient. Currently used drugs for PAH are insufficient. PAH most often occurs in young women and, if left untreated, the life expectancy is just two years from diagnosis.

How will you look to maximise the outputs of this work?

In order to maximise the outputs from our proposed work we will continue to publish our findings, both positive and negative (which is now standard practice in a number of journals such as Scientific Reports), on our website (to a lay audience), at national/international conferences and in open access peer reviewed journals. We already have a number of active collaborations in the field that will facility dissemination of our findings to the community.

Species and numbers of animals expected to be used

- Mice: 4500
- Rats: 2250

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The experimental protocols used to model pulmonary arterial hypertension are based upon well-established methods optimized to minimize animal suffering. Mice and rats between 2 months of age or older (younger animals or neonates do not show measurable remodelling of the pulmonary artery) have been shown to be highly effective model organisms to study pulmonary arterial hypertension and have validated the use of now approved drugs and helped uncover novels finding regarding the pathophysiology of the disease. Rats are required to be used in the monocrotaline-induced pulmonary hypertension, as mice are resistant to pulmonary remodelling associated with this plant alkaloid.

Typically, what will be done to an animal used in your project?

Genetically modified mice will be generated to assess the function of importance of genes thought to influence the development/pathology of lung disease, in particular PAH.
Pulmonary arterial hypertension will be induced in either GA mice or normal mice and rats by either housing the animals in a hypobaric/hypoxic chamber for 21 days chamber (similar to living at high altitude) with or without SU5416, a VEGF receptor antagonist administered by injection once weekly for 3 weeks. Alternatively PAH will be inducted in rats by a single injection of monocrotaline with PAH developing over within 14 days. Some animals will undergo ECHO MRI scanning normally on up to 2 separate occasions. Blood sampling will allow us to detect changes in hormones, endogenous ligands, circulating mediators and drug and metabolities (max 4 times per animal). PAH is characterised by remodelling of the pulmonary artery and right ventricular hypertrophy. Potential therapeutic agents or biological manipulations (e.g. genetic alterations) will be performed during these protocols to investigate if natural, synthetic substances or genetic alteration can blunt/reverse/prevent the remodelling of the pulmonary circulation and right ventricular hypertrophy. Therapeutic agents will be administered by the most appropriate route of administration/ exposure using the most suitable regime. Routes of administration may include injection via subcutaneous, intravenous, intraperitoneal, and oral routes including gavage or the addition of substances in the diet or water. If a reversal experiment is being undertaken, after pulmonary hypertension has developed (21 days or 14 days) animals will be housed for a further 21 days.

What are the expected impacts and/or adverse effects for the animals during your project?

The work will involve animals developing pulmonary hypertension. Animals housed in the hypoxic chamber show no adverse effects. The monocrotaline induced pulmonary arterial hypertension, in some cases, may induce clinical signs of pulmonary or cardiac failure in rats. These symptoms only occur in approximately 20% of animals and if seen the animals will be euthanized. Even if euthanized early the tissue from these animals will still be collected and used in experiments as they have developed the PAH.
Genetically modified mice, mice and rats developing PAH using other methods, undergoing ECHO MRI scanning, receiving potential therapeutic agents and/or blood sampling are not expected to show any adverse effects.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of the planned experiments have a mild severity. Animals housed in the hypoxic chamber show no adverse effects. The monocrotaline induced pulmonary hypertension, in some cases, may induce clinical signs of pulmonary or cardiac failure. These symptoms only occur in small number of animals and if seen the animals will

be euthanized once an appropriate humane end point has been identified (Cardiac failure occurs in 20%). We have 4500 mice and 2250 rats on the licence, so 6750 animals in total. Moderate - 20% of protocol 2 animals (250) show moderate signs that's 50/6750 so less than 1% of all animals. < 3% of rats 50/2250.

Mild – the rest.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Since pulmonary arterial hypertension is a complex multifactorial and multicellular disease we need to use animal models to fully understand the pathophysiology and triggers of the disease and the potential benefits and possible side effects of any of the targets and therapeutic agents we investigate. The experimental protocols used to model pulmonary arterial hypertension are based upon well-established methods optimized to minimize animal suffering. Mice and rats have been shown to be highly effective model organisms to study pulmonary arterial hypertension, since the remodelling of the lung and right ventricular hypertrophy are comparable with that seen in the human disease. These models have validated the use of now approved drugs and helped uncover novel finding regarding the pathophysiology of the disease. By consulting FRAME, the Journal of Alternatives to Animal Experimentation, NC3Rs, Norecopa and other resources we have confirmed that, at this point in time, no other viable alternative models are currently available for pulmonary arterial hypertension.

Which non-animal alternatives did you consider for use in this project?

Where possible, our laboratory performs experiments using cultured human pulmonary artery smooth muscle cells, endothelial cells and fibroblasts isolated from patients with normal pulmonary pressures and those with pulmonary arterial hypertension or commercially available cell line to help dissect the molecular mechanisms that contribute to the development of the disease. All novel targets and therapeutic agents are validated in human cultured cells before commencing any animal studies. We have considered cell culture and organelles and will routinely carry out extensive literature searches (including N3CRs, the Journal of Alternatives to Animal Experimentation, CRACKIT, Norecopa, FRAME etc) throughout the project in an effort to continually improve and refine our experimental techniques to identify appropriate in vitro replacements for these animal studies.

Why were they not suitable?

Since pulmonary arterial hypertension is a complex multifactorial and multicellular disease it cannot be fully modelled using cells isolated from the pulmonary artery and grown in culture. Cells isolated from patients with normal pulmonary pressures and those with pulmonary arterial hypertension are also a limited resource in the research community and have a finite life span in culture.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. How have you estimated the numbers of animals you will use?

Animal numbers will be determined so as the minimal number of animals will be used to produce meaningful data.

To estimate numbers we take into consideration the size of the effect we are interested in (right ventricular hypertrophy and pulmonary remodelling), the number of responses we intend to measure and the number of targets or drugs we intend to investigate over the duration of the licence. Appropriate advice will be taken from suitably qualified statisticians within the institution in order that our studies are always undertaken using the minimal number of animals but retaining appropriate statistical rigor
throughout. Previous experiments from our lab (including those from our GA colony) and others using the same animal models or drugs provide useful data on which to base an estimation animal numbers over the 5 years.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Animal numbers will be determined using power calculations so as the minimal number of animals will be used to produce meaningful data. Currently, I have access to a biostatistician for ongoing and future studies and NC3Rs experimental. Design Assistant. Online tool such as NC3R's Experimental Design Assistant will be used to design experiments. At all points we will follow ARRIVE guidelines for all of our experimental set ups.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Clear planning will ensure breeding is closely aligned with the proposed experimental plan. All pilot studies will be performed, followed by vigorous statistical analysis including p-value, effect size and experimental power, in such a way that any data generated can be added to any further study. Control tissue will be obtained from suitable (age/sex/breed) wild type animals through a tissue sharing initiative implemented by a number of PIs. We also ensure optimization of protocols for the high output of viable cells per lung.
Skills of the breeding wing technicians ensure GA colonies are run most efficiently – close monitoring of any changes to phenotype, pre-weaning loss, litter size and general breeding mothering performance constantly being tracked.
All procedures will follow robust SOPs.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The experimental protocols used to model pulmonary arterial hypertension are based upon well-established methods optimized to minimize animal suffering (animal models and phenotyping methods in pulmonary hypertension research. Pulm Circ. 4(1):2-9, 2014
). Both mice (allow for GA) and rats (allow for monocrotaline-induced pulmonary hypertension and extensive remodelling) have been shown to be highly effective model organisms to study pulmonary arterial hypertension and have validated the use of now approved drugs and helped uncover novel and clinically relevant findings regarding the pathophysiology of the disease. In addition, there are several advantages of using mice for understanding pulmonary arterial hypertension, such as the ability to easily manipulate genetic and physiological parameters.

All equipment, which will be used according to standard operating procedures, will be regularly serviced and checked. All procedures will be carried out according to robust SOPs, experienced staff will be involved in all procedures, measures including the timings of the studies. Measures will be taken to ensure animals becoming sick can be identified as soon as possible, sick animals will be humane killed.

Where possible

ear biopsies or hair sampling will be performed for genotyping analysis, instead of blood sampling, as this will give the least harm and stress to mice.
Wherever possible, animals will be group housed in same-sex groups and only singly housed if they demonstrate fighting within a cage.

Why can't you use animals that are less sentient?

Younger animals do not develop measurable pulmonary hypertension due to the small size of their heart and resistance pulmonary arteries. Use of younger animals would limit the number of cells that could be isolated per lung and yield insufficient numbers to carry out the proposed experiments. In the moncrotaline model of pulmonary arterial hypertension mice do not develop pulmonary remodelling, therefore rats are required. Insects and nematodes aren't suitable because they don't have lungs and therefore cannot model human diseases.

Terminally anaesthetised animals can be used for some outputs. For reasons (i.e. the time taken to develop PAH) terminal anesthesia is not practical for all outputs.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Appropriate observational monitoring protocols will be implemented. All staff will be trained and assessed before carrying out any procedures. If any animals show signs of heart failure e.g. 20% of the MTC rat model), the protocol will be immediately stopped and all animals humanely killed. Clinical signs of cardiovascular disease usually occur in the last 48 hrs of the protocol so experiments will be planned so this does not occur over the weekend to allow more frequent monitoring. In the case of our genetically modified animals, if any animals develop a moderate harmful phenotype and signs of suffering they will be immediately humanely killed and advice will be sought promptly from the local Home Office Inspector. Tissues will be harvested wherever possible if animals are euthanized, so meaningful data can still be collected. Ear biopsies will be performed for genotyping analysis, as this currently is the least harmful and stressful method for mice. We will closely monitor the situation for less harmful methods arising. For ECHO MRI treats will be used to guide them in the tubes and prevent stress.

Animals housed in the hypobaric/hypoxic chamber show no adverse effects and animals will be housed within their home cages with their cage mates and enrichment within the chambers. A period of 2 days allows for acclimatization to the hypoxic environment. Monocrotaline induced pulmonary hypertension, in some cases, may induce clinical signs of pulmonary or cardiac failure. These sudden onset symptoms occur in small number of animals (~20%) and if seen the animals will be will be euthanized Even if euthanized early the tissue from these animals will still be collected and used in experiments as they have developed the PAH. It is unclear why a small percentage of animals develop pulmonary or cardiac failure after a single dose of monocrotaline. Weight loss will be monitored to identify if this may predict the adverse effects. Furthermore, experiments will begin on a Thursday to allow close monitoring nearer the end of the 14 days, when these adverse effects usually occur. Since stress can trigger heart failure in the monocrotaline-treated rats, we will try to handle them more often to reduce stress when cages are cleaned or animals moved. Scoring sheets will be used to monitor health and wellbeing of the animals during the protocols.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All experiments will follow ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, which allow for the correct reporting of research using animals, maximising the information published and minimising unnecessary studies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I will continue to keep up to date with ARRIVE/PREPARE guidelines and receive monthly updates from the NC3Rs on events and publications regarding knowledge exchange and evidence-based changes in policy, practice and regulations. Furthermore, I will attend local training events.

Home Office

NON-TECHNICAL SUMMARY

209.Understanding brain and behaviour during neurological dysfunction in sheep

Project duration

1 years 2 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Huntington's disease, transgenic, sheep, behaviour, naturalistic

Animal types	Life stages
Sheep	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To identify non-invasive biomarkers of neurological dysfunction that can be used to track the onset and

progression of measurable changes in behaviour and circadian rhythms in a sheep model of Huntington's disease

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There is a large unmet need in the treatment of neurological diseases such as Huntington's disease (HD). HD is a late onset progressive neurodegenerative disease that causes disease in everybody carrying the gene. The disease affects the brain and causes problems with movement, thinking, planning, and emotional behaviour. Patients typically die 15-25 years after the disease starts. To track the progression of the disease or to determine if treatments are working, we need a way of measuring dysfunction. It is particularly important to focus on the earliest stages of disease, since it is likely that in the future this is when preventative therapies will be started. There is only one licenced treatment available for HD that targets the movement abnormalities. There are none that can prevent the onset or progression of HD.

The HD sheep is a good model for studying the earliest stages of HD since they carry the gene mutation that would cause disease in a human in late childhood. The HD sheep shows no overt signs of disease up to the age of 5 years, but by 5 years show some measurable changes in behaviour and metabolism. We will continue to study a cohort of HD sheep from 6 years onwards. This period moves towards the period that the HD sheep might be expected to show signs that herald the beginning of the disease. If we can identify a biomarker that changes measurably with the course of the disease, this can be used for testing potential therapies, such as gene therapies that are currently under development. For example, if a biomarker is reduced or slowed by a treatment, it shows a therapy is effective; if it gets worse or appears earlier after treatment, then that treatment may have harmful side effects. Unlike small molecule therapies that can be reversed by stopping taking the treatment, gene therapies are delivered directly into the brain. There are antisense oligonucleotide gene therapies currently under development that are being tested for safety. The effect of these will last for months. Other therapies in the pipeline that are awaiting preclinical testing, including some of those delivered via viruses, will last a lifetime. The long term effect of these therapies is not known.

What outputs do you think you will see at the end of this project?

At the end of this project we will have a comprehensive understanding of the natural history of the disease phase that occurs before symptoms appear (presymptomatic) and the beginning of the phase when symptoms may start to appear (phenoconversion period) in the HD sheep. This will add to data that are already available for the HD sheep up to 5 years of age. The expected outcome will be a complete characterisation of neurological changes, that are detectable in the circadian, cognitive, and sleep behavioural domains, in the HD sheep from 6 to 11 years. These new data will be published in peer reviewed scientific journals. This will extend our understanding of the natural history of the HD sheep model to cover the whole period of the HD sheep life when it is likely that they will be used in pre-clinical studies for testing therapies, for example.

Who or what will benefit from these outputs, and how?

A comprehensive characterisation of the HD sheep will allow future users of the model to design experiments aimed at investigating either the safety and efficacy of novel therapies without needing to first characterise the model. This is similar to the situation in 1995 when the first HD mouse was developed but not characterised. Once the natural history of the sheep model is characterised, it will increase the opportunities for using this as a model to understand the mechanisms underlying HD. In particular, knowing the time course over which HD-related changes appear will help future investigators to pinpoint the most relevant time period of study. For example, investigators interested in understanding the parts of the brain that cause loss of motor control in HD would be able to choose to study HD sheep close to the time when movement abnormalities appear.

How will you look to maximise the outputs of this work?

I will collaborate with other scientists by sharing our primary data. Knowledge will be disseminated by publication of data and/or presentation of data via lectures. We routinely publish negative results as well as positive findings. Together such data allow a comprehensive understanding of multiple aspects of the natural history the HD sheep.

Species and numbers of animals expected to be used

- Sheep: Up to 21 adult sheep

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Sheep are particularly suitable animals for this study for the following reasons

1. Adult sheep exhibit complex behaviours that are relevant to HD and that can be measured in the laboratory.

2. Sheep have large brains (that are smaller than a human brain but slightly larger than the brain of an adult Rhesus macaque monkey), with human-like anatomy, including a convoluted (folded) cortex and relevant parts of the brain (such as the striatum that is the first part of the brain to degenerate in HD).

3. Sheep are much easier to manage than monkeys, and unlike monkeys can be kept under naturalistic conditions where they are free to express natural behaviours.

4. Sheep can be safely group housed with their social companions when they are not being used for experiments.

5. Sheep are long-lived and can live for at least 14 years. This give us a much bigger window of opportunity for studying them, particularly compared to mice and rats that typically only live for a couple of years. HD sheep have measurable differences compared to normal sheep by five years of age but live for at least 12 years without overt signs of disease. They are ideal for studying the stage of HD when a patient transitions from looking normal to showing symptoms.

6. Sheep are a less sentient species than non-human primates.

Typically, what will be done to an animal used in your project?

The ability of sheep to learn and remember a task and to navigate a maze will be measured once or twice a year. Eye movement control will be assessed regularly, at least twice a year. They have all been trained to sit in veterinary slings, that will be used for measuring their eye movements since the equipment is delicate. (Eye movements are measured using a specialised camera). The slings are canvas supports that fully support the body of the sheep with its feet off the ground. This makes the sheep very comfortable and calm. The training is required to teach the sheep to stand over the sling so they can be lifted. Some measures of health and well-being will be monitored regularly throughout the year (core body temperature, heart rate). All of these will be measured non-invasively. The sheep will live together in a flock, either in pasture or in pens in barns. If they are housed outdoors, they follow the investigator from the field to the testing area where they wait until it is their turn for testing. Their participation in learning and memory tasks is voluntary, and they can end the task at any time. After testing, they return to their paddock or pen.

What are the expected impacts and/or adverse effects for the animals during your project?

Behavioural testing is non-invasive.
Adverse effects from carrying the HD gene are not expected, since it is already known that HD sheep can live for at least 12 years without showing overt signs of disease.
Because the sheep will be ageing naturally, expected adverse events are associated with this. Age-related changes may include loss of teeth, joint degeneration, slower recovery from accidental injury, stiffness or slowness

of movement. Difficulties in feeding from natural loss of teeth will be overcome by replacing their usual food with one that requires less chewing.
HD patients lose weight as their disease starts, and weight loss associated with the aging or the disease might be expected.
Their ideal body weight is taken as their weight at 4 years (fully grown) when they were acquired, when they all had good body condition scores. Up to 10% of sheep adult body weight might be expected once they become symptomatic.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Normal sheep
Behavioural testing and non-invasive physiology recording will be mild (100%), the effects of aging (50%) will be moderate.
HD sheep
Behavioural testing and non-invasive physiology recording will be mild (100%), the effects of aging and onset of HD (100%) will be moderate.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Rehomed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Measuring changes in complex behaviours (such as cognitive function, social behaviour, control of eye movement) caused by long-term expression of the HD gene requires an intact awake 'behaving' animal.
Behaviour cannot be measured in tissue culture or by using tissue isolated from an animal.

Which non-animal alternatives did you consider for use in this project?

There is no non-animal alternative for this study.

Why were they not suitable?

N/A

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken

to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Previous behavioural studies that 8-10 sheep will be needed per genotype group. The longitudinal testing means that the animals serve as their own controls.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The experiments planned can be done with this minimal number of animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use all of the sheep we have available.
If we have to kill humanely any of the sheep before the end of the study, we may collect post mortem tissue that could be shared with other groups.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use a genetically-altered sheep model of HD. This is the only sheep model of HD available.
None of the methods we will use will cause lasting harm to the animals.

Why can't you use animals that are less sentient?

HD mice do not live long enough for the proposed studies to be conducted. Anaesthetised animals cannot perform behavioural tasks.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The methodology for the studies has been designed to minimize welfare harms for the animals. Positive reinforcement will be used in our behavioural tasks, and for most of the tasks. Experienced, familiar handlers will conduct all of the experiments. For experiments where animals are required to be still (e.g. during eye movement measurement) the animals have all been trained using positive reinforcement to sit quietly in veterinary slings.
We will constantly review all procedures before the start of experiments as part of our study plan. We will update our protocols according to new information as it arises where it does not compromise the analysis of data already collected.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We already use the ARRIVE guidelines and will continue to do so. We will design any experiments for which a method does not exist using the PREPARE guidelines.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will attend relevant courses and lectures. We will use our establishment's 3Rs search tool and consult 3Rs websites for relevant advances.

Home Office

NON-TECHNICAL SUMMARY

210. Understanding cancer biology and therapeutic options in preclinical models of cancer.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

cancer, therapy, genetic models, transplantation models, metastasis

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim is to carry out fundamental cancer biology and translational research to deepen our understanding of the processes that cause cancer, and to use the most relevant mouse models of cancer to investigate new and improved therapies that will benefit patients.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

Cancer is a major health issue for the population and accounts for more than one quarter of all deaths in the UK while being a significant burden on the NHS. Understanding how cancers arise, grow and spread is paramount to our ability to prevent and treat the disease.

What outputs do you think you will see at the end of this project?

Projects covered by this licence will lead to new knowledge in fundamental cancer research and increase our understanding of how tumours initiate, grow and spread to other organs. In particular we will tease apart how cancers are influenced by their environment and metabolic dependencies.
Our goal is that these projects will identify new pathways for therapeutic intervention and lead to the development of novel anti-cancer therapies.
Work arising from these studies will be published in peer review journals and presented at national and international meetings to disseminate knowledge (to scientists and clinicians). We will also publicise our results to the public at open evenings, social media, and on our website.

Who or what will benefit from these outputs, and how?

The knowledge gained from these studies will be of interest to the scientific community (short term) and cancer clinicians. Ultimately we hope this will lead to new therapeutic approaches benefiting cancer patients (medium to long term). Furthermore, studies and validation in our preclinical mouse models should inform clinical trial design (medium to long term).

How will you look to maximise the outputs of this work?

We will continue to collaborate with national and international colleagues and disseminate this work through publication and social media at the earliest opportunity.

Species and numbers of animals expected to be used

- Mice: 75,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Cancer is a disease that results from gene alterations (mutations and loss of normal genes) and therefore the best

way to model the causes of cancer is to use genetically altered animals in which the same gene changes are recapitulated in the mouse. These genetically altered animals (GAA) are predisposed to developing cancer and act as a tool for us to test the principle of novel therapeutic strategies that could be used for patient benefit. We also use transplantation models whereby we can transplant tumour cells into specific sites of the mouse (e.g. breast cancer cells into the mammary gland) to understand how the tumour, the host environment and therapeutic agents impact upon tumour progression. Cancer is an age-related disease and sometimes we have to age our cancer-prone mouse models (for up to 2 years) to mimic the clinical situation.

Typically, what will be done to an animal used in your project?

Animals with different gene alterations will be bred to achieve test subjects which may be predisposed to cancer. Approximately 70% of the mice will not show any adverse effects relating to their breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be humanely killed when they are no longer required for breeding. It is unfortunately necessary to breed so many animals to create the right combination of patient relevant gene changes in a few key cohort animals. In the future we hope to be able to circumvent the need to use so many animals but at the moment this is the best way for us to study the causes of cancer.
A proportion of animals (no more than 25%) will develop cancer because of their genetic makeup or because tumour cells have been implanted and allowed to grow. Tumour cells may be implanted into some tissues (such as mammary gland, kidney) using a surgical procedure with appropriate anaesthesia and analgesia. Administration of an inducing agent to switch on/off particular genes may be done which only causes momentary discomfort but reduces off-target effects in other tissues.
Animals will be monitored closely by highly trained staff for well-established clinical signs such as weight loss, swelling of the abdomen, and development of visible or palpable tumours. Some of these animals (15-20%) will be given anti-cancer treatments, changes in their diet, antibodies to reduce certain immune cell types, or cancer causing agents (for example chemicals/irradiation) and the response to these treatments monitored. All animals on treatment will be closely monitored and may be blood sampled to follow changes in biomarkers which should cause only mild handling stress and momentary discomfort or may be imaged by ultrasound, CT scan or for reporter markers. Any animal that displays signs of illness such as weight loss of 20%, immobility or ruffling of the coat will be humanely killed - this depends on the type of cancer but usually within 12 months and never longer than 24 months. At the end of the study all animals will be humanely killed and tissues collected at post-mortem to gather as much information from the study as possible.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals will be predisposed to tumour development. We are very familiar with the clinical signs associated with these tumours and all researchers are trained in these models. Any animal exhibiting clinical signs of cancer will be humanely culled at pre-determined end-points of no more than moderate severity.
Anti-cancer treatments can cause gastrointestinal and haematopoetic disturbances as they do in cancer patients. Animals are monitored closely when undergoing such treatments and treatments stopped or animals killed if these effects cause suffering to the animal that cannot be resolved.
Surgical techniques are controlled with anaesthesia and analgesia and any animal who does not fully recover from such a procedure will be humanely killed.
For some of our models we use the FVB/N background strain which has a predisposition to sudden death syndrome (overall in our colonies in the last 18 months =1.1%) and neurological disorders (e.g., hyperactivity, spinning). We will periodically report the incidence if greater than 1% and will continually review the incidence of unexplained deaths. Any animal with hyperactivity or spinning that doesn't resolve (< 30 minutes) or which recurs will be killed by a Schedule 1 method.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most of the animals on this project (70%) will have a subthreshold severity meaning they will show no harm. About a quarter of animals (25%) will develop cancer in which 10% will have mild symptom such as small palpable tumours or taken at a timepoint prior to clinical signs manifestation. The remaining 15% will exhibit moderate

clinical signs associated to their cancer burden.
With respect to other procedural burden (e.g. imaging, anti-cancer drug treatment), 20% of animals will experience one or more procedures which will never exceed a moderate severity and where we anticipate 8% will experience a mild procedural severity with 12% experiencing a moderate procedural severity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Although many aspects of cancer research can be conducted using cell lines, the potential of a putative cancer causing gene and the accumulation of secondary events necessary for a cancer to fully form often needs to be assessed within the context of the whole animal. Furthermore, non-animal alternatives cannot totally model the complexities of cancer development as it is well recognised that the immune system, energy requirements and the tumour micro-environment play an important role in disease progression. Whilst the mouse is still not perfect for modelling a human being, we know that we can get closer to patient relevance using a living mouse model alongside our lab assays.
Most cancer patients die not from their primary tumour but when it spreads to other organs and to model this in the most effective way requires a living organism. Finally we know that cancer cells respond differently in the lab to anti-cancer therapies as they do in the context of the animal and so testing the efficiency of such therapies requires a complete animal system.

Which non-animal alternatives did you consider for use in this project?

Our mouse experiments are an extension of solid lab-based observations and only progress using mice when sufficient rationale is obtained based on *in vitro* cell culture. Where possible we use 3D organoid based systems, patient material and mathematical models alongside our mouse models. Organoids and 3D models are excellent ways to study genetic changes in a cancer cell and how this might affect cell growth and cell survival. We are also developing better ways to assay how cells invade and spread (as a model for cancer metastasis). We use these systems to check selectivity and specificity of a new compound for example, which allows us then to prioritise the best candidates to take forward in a physiological system for validating tumour targeting effects.

Why were they not suitable?

Modelling cancer and how cancers spread to other organs in anything other than a whole animal has obvious limitations. The interactions between the different cell types that make up a cancer (tumour cell, immune cells, blood vessels) is difficult to do in a dish. Patient samples tell us about end stage tumours but to understand what is happening at early stage of disease requires model systems.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This licence will be used by several different research groups and the numbers reflect the need for many different lines of investigation. Cancer is a disease in which several different gene changes occur and so we have to breed many mice in order to achieve the 'sweet-spot' of gene alterations in any one animal. Numbers are calculated based on our experience using the same models, published literature and advice of our in-house statistical experts.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

When planning our experiments we use pilot studies in the first instance, to inform on how many numbers we require taking advice from our in-house statisticians and using the NC3Rs Experimental Design Assistant or other statistical websites which informs us of our effect size in order to guarantee our studies are meaningful.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We share animals between experimental groups where possible - e.g. when we need normal animals for controls, we can often obtain these from our breeding colonies where they would normally not be needed in a study. We also harvest surplus tissues from experimental cohorts to use these tissues in other studies replacing the need to undertake additional cohorts.
We maximise our breeding strategies to generate the most effective breeders to create the genetics of interest for our studies and we use tumour transplant models where appropriate, which do not require breeding of genetically altered animals and thus use fewer animals in total per study.
We use inbred strains of mice which are nearly identical to each other resulting in less variability between animals and allow us to use fewer animals to achieve a statistically significant result.
To reduce numbers of mouse-based experiments we always perform studies using cell lines or 3D models so that only our strongest hypotheses are tested in the mouse.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use mouse models with the same genetic changes that are known to cause human cancer – so accurately replicating the human disease. These genetic changes are specifically altered in the tissue of interest so that unrelated effects in other tissues do not occur. All animals are monitored regularly for signs of normal behaviour and are humanely killed if they exhibit moderate adverse symptoms. All staff are expertly trained in these clinical signs. Regular monitoring of mouse welfare allows us to complete studies at the earliest endpoint in which we observe a significant result to prevent unnecessary suffering resulting from high tumour burden.

Why can’t you use animals that are less sentient?

The mouse is a mammal and warm-blooded which shares many features of human physiology and metabolism not found in other cold-blooded species such as flies and worms. Furthermore with the ease of manipulating the genetics of the mouse, this makes the mouse the best model organism to understand the genetic changes observed in cancer.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals are housed in a dedicated facility proactive with environmental enrichment and receive anaesthesia and analgesia as appropriate.
All animals are health checked daily in addition to the routine monitoring by the researcher. Where any animal shows abnormal behaviour this animal is placed on enhanced inspection. Study animals are often weighed and tumours measured at regular intervals to detect early clinical signs.
We have written standard operating protocols for all our models with researchers being trained and signed off only when proficient in the clinical signs of the models.
We always refer to previous studies for adverse effects of anti-cancer therapies and when a group is given a treatment for the first time, we initiate the study with a small number of animals (n=3-6) which is closely monitored before extending to a larger number. Where possible we use the least invasive method of drug administration.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We adhere to the Workman Guidelines for the welfare and use of animals in cancer research and regularly review the NC3Rs website.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We continually review our processes and take advice from the Named Veterinary Surgeons, Named Training and Competency Officer, Home Office Inspectors and the NC3Rs website. Our technical staff are very proactive in adopting 3Rs advancements such as non-aversion handling and single-use needles and contribute/attend in-house events such as 'culture of care AWERB' and 3Rs workshops.

Home Office

NON-TECHNICAL SUMMARY

211. Understanding how behaviour modulates neural responses in the visuomotor system

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Brain activity, Vision, Decision-making, Internal state, Motor activity

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We have two overall aims. First, we want to understand how the brain integrates visual information with information about the subject’s behaviour and internal state. Second, we want to understand how the brain uses this integrated information to control decision-making and motor outputs.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration

of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our understanding of how the brain enables us (and non-human animals) to sense the environment, make good decisions, and act accordingly is still very limited. One problem is that a large portion of brain activity cannot be explained by sensory input. For example, the exact same image or video can elicit different activity every time it is seen, even in nerve cells that are in the eye and sense the incoming light.
Using the nerve cells in the early visual system as a model, this project aims to understand:
(1) how activity in nerve cells is influenced by non-sensory factors such as the animal's behaviour or internal state,

(2) which biological mechanisms underlie these influences, and

(3) how the modulation of this sensory brain activity affects brain activity controlling decision-making and motor actions.

This project will lead to a better explanation of the activity in the brain, a better understanding of the computations performed in the brain, and thus a better understanding of the brain's failures in disease.

What outputs do you think you will see at the end of this project?

The main benefit of our research is to increase knowledge about brain function. We currently know little about how the brain works. In our field of research, scientists only recently realized that the visual system, i.e. nerve cells and brain areas that make sense of the visual input, is affected by behavioural state like locomotion and the level of alertness. For example, neurons that reliably respond to a certain visual input like the picture of a black bar on white background respond more or less to this very same stimulus depending on whether the animal is highly alert or tired and drowsy. That even nerve cells in the retina are affected by this modulation is a very recent finding from our previous work. What we do not yet know are the specifics of the behavioural modulation of vision, i.e. whether the processing of certain stimuli is more or less modulated, or whether certain types of cells are more or less prone to modulation by behaviour. We also do not know why the brain modulates visual signals depending on behavioural state, and how does it do so.
The goal of this project is to contribute the following milestones:

- Understanding the effect of the animal's behavioural state like its speed of locomotion or its level of alertness on visual processing
- Understanding the purpose of this modulation regarding the efficiency of visual processing and regarding behavioural performance in tasks that depend on vision

- Understanding how these behavioural effects are mediated

At the end, we will have a better understanding of how behaviour and internal states shape vision.
In addition to new knowledge about the brain, we will also contribute by developing techniques to learn about the brain. We will refine methods to record functional activity of single retinal ganglion cells (neurons that transmit information from the eye to the brain) over a long time period of weeks to months.
The knowledge we gain will be published in peer-reviewed articles. Manuscripts will also be published on a preprint repository so findings are available to public as soon as we deem them ready for publication. We will present findings at conferences and during invited talks.
Datasets of neural recordings and simultaneously recorded behaviour of animals will be published online, and code developed by our group to analyse the data will be made publicly available.

Who or what will benefit from these outputs, and how?

The outputs of this project will directly benefit scientists who study the visual system and the processing of visual information in the brain, and scientists who study decision making based on visual information. In addition, findings on behavioural modulation in visual processing are likely to generalise to other sensory modalities. So, this work may lead to new hypotheses in these fields.
The outputs can also directly benefit scientists working on therapies of eye diseases because we are now able to

record the activity of single retinal ganglion cells in awake animals on a daily basis over a time span of weeks up to several months. This technique could be used by scientists developing therapies to treat retinal degeneration as they can observe the functional changes of retinal ganglion cells throughout their therapeutic treatments. Moreover, the outputs of this project may have indirect benefits for scientists studying visual impairment in relation to diseases that are accompanied by abnormal behaviour. For example, ADHD is thought to involve the malfunctioning of a visual area (superior colliculus) involved in guiding our attention. It is thought that this area is overactive in ADHD patients, and therefore these patients cannot stabilize their attention but get distracted by any new visual stimulus that is perceived. Our goal to better understand of how visual signals in the brain are influenced by behavioural states or by neuromodulators that mediate these states may lead to a better understanding of visual malfunctions in disease. We expect that these benefits will only be seen in the long-term and after this project is concluded.

How will you look to maximise the outputs of this work?

To maximize the outputs of this project, we will closely collaborate with colleagues at our establishment who study the visual system or particular behaviours in mice. We will also promote the publications of our articles and datasets on social media channels like Twitter and via the media office of our establishment.

Species and numbers of animals expected to be used

- Mice: 2,100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Brain architecture of mice and particularly the visuomotor system that we will study is very well conserved across mammals and is thus similar to that of humans. Our findings will thus have a high probability to generalise to other mammals and humans.
Mice use vision in their natural habitat and they can be trained on tasks that require vision. Using mice, we can study visual processing in the brain and how changes in this processing influence behavioural performance.
Many genetic tools exist for mice. These tools enable us to study specific cell types, and measure and manipulate neural activity.
Mice can be kept in animal facilities typically provided at a university setting under conditions that cater their well-being and health.
We will be using adult animals because we are studying fully developed brain function.

Typically, what will be done to an animal used in your project?

Almost all of our mice will be bred in the local animal facilities.
Mice that will be used in experiments (an estimated 20%) usually receive an implant attached to their skull so they can be head restraint. We will gain access to their brain by removing a small part of their skull (about 0.5 – 5 mm in diameter). In some mice, we will induce the genetic expression of certain molecules by injecting viral vectors into the brain or the eye. We may also implant light guides (tens to hundreds of microns in diameter) to optically access deeper parts of the brain. These interventions are necessary to measure neural activity, identify certain cell types, or manipulate neural activity. These procedures are all performed under full anaesthesia and under aseptic conditions, and animals will be treated with analgesia during recovery from the surgery. Several surgeries may be necessary to accomplish our goals, for example if it is better for the animal’s well-being to split one surgery into

two or when genetic expression needs to be induced at certain time points after the animal has been trained, where training necessitates the head restraining implant. No animal will undergo more than 5 surgeries (each lasting more than 10 min).
Mice are then acclimated to head restraint over several days before we perform experiments to record neural activity. These experiments last at most 4 hours a day. Depending on the method of recording, the experiments continue for several days up to several months.
Some animals will be trained to perform a specific task, e.g. they have to choose one of two visual stimuli. To keep the mice motivated to learn and perform the task, their daily water intake will be restricted, and they will be rewarded with water when performing the task correctly. Animals are monitored every day for signs of dehydration, weight loss and abnormal behaviour, and their water intake will be adjusted accordingly.
At the end of the experiments, the animals will be culled or perfused for further histological processing of their brains.

What are the expected impacts and/or adverse effects for the animals during your project?

Animals will undergo surgery under general anaesthesia but are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. None of the surgical intervention, implants or viral injections is expected to lead to long-term harm.
Head restraint will cause stress to the animal but usually only during the first few sessions when the animal is acclimated. Once the animal is used to the head restraint, most of the time animals do not show signs of stress and behave normally, e.g. they run on a treadmill or they groom themselves.
In some experiments, in which neural activity is recorded, the insertion of the recording probe (a few microns in diameter) may cause temporary pain when the dura, i.e. the skin surrounding the brain, is penetrated.
Under water restriction, mice will most likely experience weight fluctuations and may show signs of dehydration. These effects will be monitored every day and provision of water will be increased as necessary.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

About 95% of the animals (2,000 of 2,100) are expected to undergo Protocol 1 (Breeding) and will experience a severity category of mild.
20% of the animals (420 of 2,100) are expected to undergo Protocol 2 (Measurements and manipulation of neural activity during behaviour), and thus will experience a severity category of moderate.
What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We will study how the brain processes visual input during different behaviours, and how this visual processing impacts decision making and motor output. No artificial or non-living biological model system can replace the animals we use to perform our studies as we do not know the neural architecture and function that performs visual processing and controls behaviour.

Which non-animal alternatives did you consider for use in this project?

Parts of the brain (e.g. brain slices) and the eye (in particular the retina) can be removed from a dead animal and kept under conditions that allow nerve cells to stay functional. Such experiments are termed “in vitro” studies. These model systems can be used to study circuits of nerve cells and to some degree their function, e.g. which nerve cells are active together.
Artificial neural networks offer the opportunity to simulate biological neural networks.

Why were they not suitable?

In vitro models of populations of living nerve cells lack the input from the rest of the brain and they cannot be studied in relation to the animal’s behaviour, which is an essential goal of this project.
Regarding artificial neural networks, we have too little information about the biological networks in order to reconstruct them artificially. More importantly, we do not understand how nerve cells react and interact during natural behaviour.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Using statistical methods, we have determined how many nerve cells we need to record to answer each of our scientific questions and to draw robust conclusions from our results. Based on our previous experience, we have estimated how many nerve cells we will record from each animal. Both numbers together, i.e. the number of necessary nerve cells and the number of nerve cells recorded per animal, provide an estimate of the number of animals we will need to use for this project.
Several factors complicate the estimation of the numbers of animals though. First, there are uncertainties on the actual number of nerve cells we can record from each animal, as this depends on the quality of the preparation and the recording, as well as individual differences across animals. Second, although we have planned a number of specific experiments that we want to perform, the exploratory nature of our research demands some degree of flexibility in the planning of experiments. In the case of highly unexpected results from one experiment, we may adjust subsequent experiments, which may lead to different numbers of animals needed. In the case of inconsistent results from performed experiments, we will perform further experiments on other animals if it is likely that these experiments will lead to consistent and robust results.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Our experimental technology yields a large number of nerve cells that we are able to record in each animal: (1) we use state-of-the-art technology that allows simultaneous recording of hundreds to thousands of nerve cells; (2) we can perform these recording sessions several times on the same animal, so that every session yields another population of recorded nerve cells.
Our longitudinal experiments on awake animals last from several days to a few months. To gather the largest possible amount of data from each animal, and thus reducing the total amount of animals, we need to pay

particular attention to the health and well-being of each animal. Please, see Refinement for details on this.
When we are investigating functional differences between specific types of nerve cells, we will use several methods of targeting several types within the same animal. For example, we can use a transgenic animal to target one genetically defined cell type. At the same time, we can define other cell types based on the anatomical connection to other brain areas by injecting tracers into several brain areas.
We will constantly review the methods we are employing by following published literature or receiving advice from other experienced researchers in order to increase the effectiveness of our methods. This will reduce the number of failures, for example in targeting specific cell types, and will increase the success rate of experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Efficient use of animals bred both under this licence and under the authority of other licences within the establishment that allow for the breeding and maintenance of mice expressing genetically encoded reporters and effectors of neuronal activity. For example, we will avoid ordering additional wild type experimental animals whenever surplus mice can be obtained locally.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In our experiments, we will use purpose bred mice, some of which are genetically altered so we can distinguish and target specific types of nerve cells. In comparison to many other mammals, particularly larger animals, mice can be kept in the laboratory under conditions that cater their health and wellbeing very well. The genetic modifications in the mice we are using are not expected to lead to pain or any harm for the mice. All animals will be co-housed with litter mates as mice are social animals. We will only make exceptions to this rule if the animal's health is impacted, e.g. when animals get injured by other cage mates or when animals are too vulnerable due to surgical implants or sutures.
We will perform surgeries to access parts of the animal's brain and to implant a plate that allows us to head restrain the animal. We always use appropriate anaesthetic and analgesic regimes for pain relief during surgery, and surgeries are performed under aseptical conditions. The implant weighs less than 1 g and does not affect behaviour of the animal in a negative way.
We will head restrain the animal during our experiments so we can reliably measure the activity of many neurons while also controlling the animal's sensory input, e.g. via visual displays. We will acclimatize the animal being head restrained step by step, starting by simply handling the animal and then restraining it just for a few minutes. After a few days, the animal is getting used to the procedure and does not show signs of stress anymore.
To record and manipulate brain activity we will use methods of two-photon imaging, electrophysiology, opto- and chemogenetics. Two-photon imaging can be performed on awake animals on a daily basis for weeks and months without any invasive procedures in addition to the initial surgery. The same is true for the manipulation of activity via optogenetics. Manipulation of activity via chemogenetics requires the injection of specific drugs that then only act on previously targeted neurons that express receptors for these drugs. Electrophysiology necessitates the insertion of a probe into the brain to record electrical activity of the neurons. The recording probes we are using are only a few microns in diameter, much thinner than a human hair. The animal might feel mild pain when the probe penetrates the skin surrounding the brain (the dura), but does not feel pain as the probe penetrates the brain itself as there are no pain receptors in the brain.
We will train some of our mice to perform a visual decision task where they have to choose the correct stimulus out of two presented stimuli. We do this to study how the brain processes visual input and it uses this information to

make decisions. To keep mice motivated to learn and perform this task, we restrict their daily water intake as they are rewarded with water when they perform correctly. We monitor the animals every day for signs of dehydration and ill health and increase their water intake accordingly.
Our research depends on the mice being healthy, cooperative, and engaged in the tasks that we trained them to perform, so we have many reasons to avoid any suffering. The measures we take to avoid any unnecessary suffering work: the mice are cooperative and engaged.

Why can't you use animals that are less sentient?

The brain is still developing until early stages of adulthood. Our goal is study processes in a fully developed brain, and we therefore use adult animals. A further advantage of using adult animals is that they do not grow anymore; implants mounted on the head therefore do not need to be adjusted for changing sizes of the skull.
Another goal is to study the influence of various behaviours on the processing of visual inputs. Therefore, we have to work with awake animals.
The function of the brain and its nerve cells is not well understood in most if not all animals. Studies of the brain are therefore necessary and useful in any animal species. We decided to use mammals, in particular mice, rather than species of a different class that may be considered less sentient, because the brain structures we are studying are conserved within mammals including humans but show greater differences across animal classes such as fish or birds.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

- After surgeries, the animals receive analgesic to minimize pain and are monitored closely for at least three days.
- Animals are acclimatised to the head restraint for several days starting with a few minutes of restraint on the first day. Animals are taken off head restraint if they show high levels of stress, and acclimation is continued on a slower rate for these animals.
- Animals under water restriction are closely monitored every day for changes in weight and signs of dehydration and illness. Water is given in higher amounts or ad libitum to restore full health.
- Training of animals on decision tasks is performed step wise starting at an easy level and slowly introducing more difficult conditions. The time point of introducing more difficult conditions is based on the performance of each individual animal. In this way, the animals are learning the task in the shortest time possible.
- Throughout the duration of this project, we will adapt all methods if better strategies become known and available.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will continuously refine our training procedures to follow best practice in this area, which is a very active one at the moment; e.g.,
Guo et al (2014) Procedures for behavioural experiments in head-fixed mice. PLoS One 9: e88678.
Burgess et al (2017) High-Yield Methods for Accurate Two-Alternative Visual Psychophysics in HeadFixed Mice. Cell Reports 20: 2513-2524.
Goltstein et al (2018) Food and water restriction lead to differential learning behaviours in a head-fixed two-choice

visual discrimination task for mice. PLoS One 13: e0204066.
Our participation in the NC3Rs working group mentioned elsewhere, through which we will disseminate any refinements throughout the community, also puts us in an excellent position to hear about and adopt any relevant updates to refinements developed by other researchers.
Surgical procedures will be carried out according to the published LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will follow several avenues:

- Participation in NC3Rs working group on 'high-yield rodent behaviour', through which we share best practice in this area;
- NC3Rs bulletins and newsletters;
- News and information provided by local NACWO/NTCO/NIO;

Scientific meetings with presentations on mouse behaviour and training.

Home Office

NON-TECHNICAL SUMMARY

212. Understanding how to stimulate the immune system to remove old, senescent cells, from aged tissues

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Ageing, Telomeres, Telomerase, Immunity, Senescence

Animal types	Life stages
Zebra fish	embryo, juvenile, adult, neonate, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim is to determine which immune cells and mechanisms we can target to improve the removal of old, senescent cells, from aged tissues, where we know they contribute to chronic diseases of old age. The ultimate aim is to identify new potential therapeutic targets to promote healthy ageing.
Specifically:
Aim 1- Identify key immune cells involved in senescence cell clearance (Years 1-2)
Aim 2- Identify mechanisms involved (Years 1-3)
Aim 3- Test ways to put it right
(Years 4-5)

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A major medical problem associated with ageing is that the different tissues in our bodies become damaged over time. A major reason for this is the accumulation of old or “senescent” cells. It is likely that, as we age, our immune system becomes defective in its ability to clear senescent cells from tissues – if we could understand what goes awry this may provide opportunities to design therapies to support this particular function of our immune system, as we age.

What outputs do you think you will see at the end of this project?

This **first output** from this work is the identification key immune cells involved in clearing old, senescent cells. This is important, as it will allow us to focus our efforts in understanding the mechanisms in these cells that may be dysfunctional with ageing, which will be our **second output**. At this point we should have a high impact publication and disseminate this knowledge to the public and peers via conferences, press releases and public talks.
The **third output** of this work is to test known and also potential new therapeutics to stimulate the immune system to promote the removal of these damaging senescent cells to promote healthy ageing. This knowledge should then be translated into one or two high impact publications and provide proof of principle, pilot data to further develop drug testing in collaboration with Pharma in a future collaborative project. As always, this knowledge will be disseminated to the public and peers via conferences, press releases and public talks.

Who or what will benefit from these outputs, and how?

The knowledge obtained from outcomes 1 and 2 should be public within the five year PPL period. Outcome 3 will likely take a bit more time, but should be made available at year 5 and 7 likely.
The benefits from this project are targeted at the healthcare of the continuously growing ageing population. This

project aims to contribute to a step change in the treatment or even prevention of chronic diseases of ageing, which we know are highly contributed to by the accumulation of these old, senescent cells, in tissues.

This project is part of a wider effort to highlight a new therapeutic strategy to clear senescent cells, using our natural defences, surpassing the non-specific and highly toxic senolytic drug strategy. Such immunotherapeutic strategies will pave the way to a step-change in the treatment of chronic diseases of ageing, with huge health and social impact in the EU.

How will you look to maximise the outputs of this work?

Besides the high impact publications that we hope to obtain from outputs 1-3, we will aim to publish any new significant method (peer-reviewed and open access) that we develop that may help the scientific community tackle the problem of damaging senescent cells in tissues. This is likely to be relevant particularly if we succeed in establishing the state of the art *in vivo* and *ex vivo* live imaging techniques we are aiming to.
I aim to publish all our work in reputable open access journals, freely available to the public.
Furthermore, there are specific journals that aim to publish all sound knew scientific knowledge, even if reporting unsuccessful approaches, to maximise dissemination of knowledge and minimise duplication of work by others.
I have on-going collaborations with other experts in the field who also have close links to Pharma and can help develop any further tests or drugs to maximise the benefits from this work. This is also likely to lead to further joint grant applications, public talks and key note lectures at international conferences.

Species and numbers of animals expected to be used

- Zebra fish: 22,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The methods for generating mutants and transgenics are optimised to minimise numbers and adverse effects are minimal. Zebrafish have a number of additional advantages for these studies, including their near transparency, genetic tractability and extensive genomic resources. My previous work has provided opportunities to develop defined end-points to my ageing protocols that will be employed in this PPL. Fin clipping for genotyping purposes will be performed whenever possible in larvae before independent feeding. Procedures will be tested first in few animals in pilot experiment (up to 3 of each experimental and control group).
I will use animals until old age or genetically altered animals that age faster. Ageing phenotypes are required since we are testing cell interactions in old tissues, testing ways to improve these interactions and see if that has a positive impact on the health of old animals or promote a healthier ageing. I will use genetically modified premature ageing models (previously established) to test the validity of specific molecules as therapeutic target to ameliorate the cell interaction we are studying, in old tissues and 2) Provide a quicker model to display some (but not all) ageing phenotypes that we know mimic natural ageing.
Premature ageing model phenotypes can be divided in early and late. Early phenotypes occur before the half-life of these animals (c.9 months of age), late phenotypes occur nearer the end point of their lives (c. 12-15 months). Because of this, I will use defined end-point criteria, previously characterised by me to determine when to sacrifice the animals to measure specific phenotypes. The time points will generally be 1, 3, 6, 9 and 12 months of age for WT and prematurely aged siblings, which will be extended to 18, 24 and 36/43 months of age in the WT setting. The later time points are important, as there are specific ageing phenotypes such as wasting (equivalent to frailty in old age in humans) , that occur only at these older ages.
Only a maximum of 20% of animals, however, will reach the old age.
We may use larvae forms before 5.2dpf (non-protected) for genotyping, as much as possible, to minimise number of animals required to grow.

Typically, what will be done to an animal used in your project?

Most animals will be used for breeding and generating transgenic and/or mutant lines. Fish will be grown to selected time-points, where they may:

- Be culled for post-mortem analysis of fixed tissues or isolated cells from selected tissues.

- Undergo specific tissue regeneration challenges and culled at selected time points post-procedure for post-mortem analysis of fixed tissues or isolated cells from selected tissues. An example challenge is irradiation. The dose we will be using has previously been shown to induce signs of illness in a maximum of 10% of animals.

- Undergo selected drug treatments aimed at improving tissue regeneration and culled at selected time points post-procedure for post-mortem analysis of fixed tissues or isolated cells from selected tissues.

Fish may also undergo non-invasive behavioural testing, where we film animals swimming, interacting with objects, which will allow us to monitor general health of the animals.

What are the expected impacts and/or adverse effects for the animals during your project?

To understand how the specific cell interaction we are studying is impaired with ageing requires animals to age, whether naturally, or in the presence of mutations that mimic premature ageing syndromes in humans. During the course of their life-span premature ageing will result in weight loss, animals may develop arched backs and cataracts. This, however, will only happen to maximum 20% of the animals used in this project, and for a short amount of time. Note however that death is not an endpoint and fish will still be is able to swim and feed prior to reaching their end-point. To ensure the moderate humane end point is not exceeded, animals will be closely monitored, particularly after the age of 9 months when weight loss is accelerated. All fish will be regularly monitored for their ability to feed, swim, socialise/behave, grow and generally thrive well compared with wild type equivalents. We will use a humane endpoint based on body condition and fish will not be kept for long periods in poor condition.
Moreover, other than in terminally anaesthetised animals dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. Aged fish may be more sensitive to regeneration assays and may not recover from anaesthesia or sometimes, even though they recover, they may display a wound that does not heal properly and will have to be culled. However death should not exceed 10%. For young fish used in regeneration testing, though, it is expected that the rate is lower.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most animals will be used for breeding and generating transgenic and/or mutant lines (Mild). To understand how the specific cell interaction we are studying is impaired with ageing requires animals to age, whether naturally, or in the presence of mutations that mimic premature ageing syndromes in humans. During the course of their life-span premature ageing will result in weight loss, arched back, cataracts (ageing phenotypes in later time points are considered moderate). This, however, will only happen to maximum 20% of the animals used in this project. Animals will be closely monitored, particularly after the age of 9 months when weight loss is accelerated. Moreover, a small portion of animals (up to 20%) will be imaged under anaesthesia post fin clipping or post skin incision (mild). Larvae may require to be immobilised (embedding in agarose) for study and this would be considered moderate. A small portion of animals will be administered with a gut damaging drug for assessing cell interaction in young vs ageing post tissue challenge (moderate). A small portion of animals will undergo irradiation to stimulate cell senescence (Moderate).

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Understanding how the different immune cells interact with different types senescent cells, how this is affected with ageing and how this impacts on the animal's health requires in vivo studies using an animal model. These are processes that depend on the interaction between different types of cells in a tissue and organismal context.

Which non-animal alternatives did you consider for use in this project?

The objectives described above are part of an integrated programme of work, including elements of in vitro and in silico work alongside the in vivo experiments. In vitro cell culture using human immune cells (e.g. neutrophils, macrophages) will be performed in parallel. Where candidate genes can be identified from previous work, or by searches of available databases of genetic information, this will be performed to replace in vivo screens. In addition, most of the candidate genes will be identified from human clinical studies, replacing the zebrafish screens. Where possible larvae forms will be used, whenever a simpler hypothesis is being tested.

Why were they not suitable?

There is only so much we can test in vitro with isolated cells. In particular, the immune cell types likely to be involved in senescence clearance are likely to require interaction with each other to effectively clear senescent cells. This is not possible to reproduce in vitro with current technology. We will be able to ask individual questions with a particular cell type, but we will not be able to determine the impact of the cell-cell interaction for the animal's health.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

I have used data generated from previous PPL to gain insight into the number of animals required per line and protocol. I established this using power calculations, based on
http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html
.
When the given protocol has not yet been tested by me, I will perform a small pilot study with maximum 3 animals per genotype as a refinement strategy. I have used data from the literature to estimate number of animals required for such new protocols. Overall, data suggest I need a maximum of 16 animals per genotype, per time point to allow statistical interpretation.

Animal Numbers

The previous PPL allowed me to determine that I will need a maximum of 16 animals per assay to determine statistical significance (see example below for gut permeability assay) **.**

I have estimated that I will require 22,500 adult WT, mutant and/or transgenic fish for the total in this PPL over 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have sufficient pilot data from all studies to perform a priori power calculations to calculate group sizes. We will not initially exceed the predicted group sizes required to detect a 25% difference with 90% power (alpha of 0.05 as per convention), and I have calculated that we need a maximum of 16 animals of each genotype per experiment to detect differences. Based on established calculations using the 3Rs recommendations.
We will increase group sizes only if greater power is required, if the variance is greater than in pilot studies, or where the biological effect to be detected is less than 25%. Whenever possible we will use the same adult fish for in vivo assays, including non-invasive, non-harmful behaviour assays, followed by schedule 1 and in vitro cell culture assays, to try and reduce the number of fish that have to be grown in parallel for the different experiments.
Furthermore, an advantage of working with zebrafish, both as larvae and adults is that we only need one animal to look at all tissues at the same time, on the same slide, which increases the quantity and relevance of the information retrieved per animal. Where possible temporal studies will be performed using the same animal to gather serial data. As part of good laboratory practice, we will write an individual study plan (ISP) for each experiment involving regulated procedures.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Many of the functional assays will be performed in vitro using isolated cells from different tissues of the same animal, allowing large amount of data to be collected from reduced number of animals
To minimise the number of animals grown to an age protected by the Act, we will try and genotype the lines before the age of 5 days post-fertilization, after which they are protected by the Act, to identify unwanted genotypes to reduce the number of adults to grow. For example, embryos may be genotyped through micro-abrasion using the ZEG system.
Opportunities to reduce the number of animals required to develop a new line may be possible as new transgenic technologies are developed (e.g. genome editing). Breeding programmes to develop the new lines and intercrosses will be developed and we will use in-house databases (e.g. labtraks) to aid our efforts to maintain the minimal number of animals per line. Where necessary, gametes are expressed either to maintain fertility or to allow in vitro fertilisation, and numbers are determined by the requirements of maintaining fertility and preserving lines.
Furthermore, whenever possible we will use the same adult fish for in vivo assays, including non-invasive, non-harmful behaviour assays, followed by schedule 1 and in vitro cell culture assays, to try and reduce the number of fish that have to be grown in parallel for the different experiments.
Additionally, using such a small sized animal like the zebrafish allows us to look at all tissues at the same time, on the same slide, which increases the quantity and relevance of the information retrieved per animal. Where possible, temporal studies will be performed using the same animal to gather serial data.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

I will use the organism with the lowest neurophysiological sensitivity possible for work of this kind, the zebrafish. The methods for generating mutants and transgenics are optimised to minimise numbers and adverse effects are minimal. Zebrafish have a number of additional advantages for these studies, including their near transparency, genetic tractability and extensive genomic resources.
My previous work has provided opportunities to develop most procedures (with the help of NACWOS and the

NVS) used in this PPL and establish defined end-points to my ageing protocols that will be employed in this PPL. Fin clipping for genotyping purposes will be performed whenever possible in larvae before independent feeding (before 5.2dpf (non-protected).
Any new procedures will be tested first in few animals in pilot experiment (up to 3 of each experimental and control group), under NACWO and NVS guidance.

Why can't you use animals that are less sentient?

I will use zebrafish as an ageing model because I have shown that, similarly to humans and in contrast to inbred lab mice strains, removing telomerase from zebrafish leads to premature ageing in the first generation. This means that zebrafish are the animal model with the lowest neurophysiological sensitivity mimicking key aspects of human ageing, required to address the scientific questions in this PPL.
Many of the objectives in this PPL will be addressed by collecting tissues from animals that have been killed at the end of the experiment/study.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The methods for generating mutants and transgenics are optimised to minimise numbers and adverse effects are minimal. Zebrafish have a number of additional advantages for these studies, including their near transparency, genetic tractability, extensive genomic resources and small size. My previous work has provided opportunities to develop defined end-points to my ageing protocols that will be employed in this PPL. In particular, my previous PPL allowed me to optimise histology, so I know that the culling methods chosen are ideal for tissue preservation, required to address the scientific questions in this project. Scoring sheets and imaging (photographs) will be used to further refine the end-points. Fin clipping for genotyping purposes will be performed whenever possible in larvae before independent feeding.
Procedures will be tested first in few animals in pilot experiment (up to 3 of each experimental and control group).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will follow the latest relevant literature and in house expertise as well as 3Rs resources and guidelines for zebrafish (https://norecopa.no/media/7384/zebrafish.pdf)

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I attend departmental meetings on a regular basis where all researchers, technicians, NACWOs, NVS, NIO and NTCO share relevant information regarding recent advances in the 3Rs. In addition, I will also meet with representatives from organisations such as NC3Rs to keep abreast of the latest developments that can be applied to zebrafish studies.

Home Office

NON-TECHNICAL SUMMARY

213.Understanding mechanisms of immunotherapy and the basis of immune related adverse events (irAEs)

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to better understand how immunotherapy works and how immune related adverse events (irAEs) are precipitated. irAEs limit the use of otherwise highly potent drugs that result in beneficial clinical outcome for the treatment of immune mediated diseases ranging from cancer to autoimmunity.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished. Why is it important to undertake this work?

By addressing the knowledge gaps underpinning the mechanistic basis of immunotherapy and irAEs, we can minimise the risk of certain adverse drug reactions and identify strategies that can be used to reverse them. This will also allow new drug candidates with the potential to cause an irAE to be identified and rejected at an earlier stage of development, rather than progress to patient trials and or wider clinical use.

What outputs do you think you will see at the end of this project?

The primary output of this work relates to new knowledge and information about the mechanisms by which immunotherapy works and the reasons why see adverse effects. This will be achieved through understanding the mechanisms of current immune targets, refinement and development of new treatment and/or reagents based upon a better understanding of the host immune responses involved. We will also publish our findings in peer-reviewed journals, present at scientific conferences and
participate in public outreach activities. Primarily, our data will be of interest to scientists, pharmaceutical companies and clinicians. Principles established during these studies should also be applicable to immunotherapy against infectious diseases for both clinical and veterinary applications.

Who or what will benefit from these outputs, and how?

The ultimate aim is to develop and/or improve treatment options for patients with a range of immune mediated diseases. Potential benefits arise from informed design of combination treatments in which monoclonal antibody (mAb) are given alongside conventional and other emerging modalities including other immunotherapy. Consequently, if applied appropriately, unlike conventional treatment, new immunomodulatory therapies should be more potent, and not be associated with long-lasting toxicity.

Knowledge generated from this project has the potential to benefit patients, clinicians, industry and medicines regulators (long terms, 5years+) by:

- Identifying novel strategies for overcoming tumour immunosuppression with the aim of identifying novel treatment approaches for application in patients.

- Allowing new drug candidates with the potential to cause an irAE to be identified and rejected at an earlier stage of development, rather than progress to patient trials and or wider clinical use.

- Developing novel screening procedures to identify patients that are predisposed to a particular irAEs, and characterising biomarkers that enable the earlier identification of patients experiencing an irAE, thus limiting their exposure to the drug and informing clinical diagnosis and prognosis.

A secondary benefit relates to the training of the next generation of immuno-oncologist and drug safety scientists, who will go on to lead future research in this area and/or occupy decision-making positions in industry/regulation (short term, 3 years).

How will you look to maximise the outputs of this work?

We will publish in peer-reviewed journals, present at scientific conferences and participate in public outreach activities. Primarily, our data will be of interest to scientists, pharmaceutical companies and clinicians. We are open to collaboration with academic researchers and scientists from industry/pharma in terms of sharing reagents and data. The proposed project will cultivate a programme of work that builds significantly on the antecedent research in relation to antibody immunotherapy, and offers the promise of exciting collaborative work where all collaborators will benefit from the extension of knowledge generated from this work.

Species and numbers of animals expected to be used

- Mice: 600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are investigating the in vivo effects of immunotherapy with a view to establish their mechanisms of action and their adverse effects, with therapeutic application in humans. These interactions occur between different immune cells, organs and tissues, and as such cannot be reproduced in vitro. There is a remarkable consistency between the immune cell types and gene expression profiles in mouse and human immune system. Majority of gene expression patterns- conservatively estimated at 80 percent– are the same in mouse and human, and this also suggests a role for transcriptional regulators that may underpin these similarities. Furthermore, the ever-increasing availability of genetically manipulated mice has allowed genetic dissection and meaningful modelling of human disease. Mice will be used for this study since they are the least sentient species of mammal that have the following characteristics: 1. The cellular and molecular interactions of the mouse immune system are broadly similar to those of humans, allowing us to investigate relevant immunological strategies and mechanisms; 2. Individual mice within a given inbred strain are considered genetically ‘identical’, thereby reducing variability and allowing valid conclusions to be drawn from experimental data; 3. Numerous models of tumour and autoimmunity have been established and characterised in mice that are strain specific; 4. Numerous strains of genetically altered mice have been developed, such as those expressing antigens of interest or genes of interest removed. For majority of this project we will be using mice that are 6-12 weeks age, which is when they have a fully developed immune system. For some studies, where we investigate how drugs affect the early development of immune tolerance and immune homeostasis, will use mice that are 4-6 weeks old, where the immune system is not fully developed.

Typically, what will be done to an animal used in your project?

Mice will be administered with substances or combination (immune cells, tumour cells, disease inducing compounds, drugs- monoclonal antibodies, pharmacological inhibitors, small molecule drugs, biologics, disease inducing chemicals) through a variety of routes (subcutaneous, intradermal, intramuscular, intravenous, intraperitoneal, intratumour, mammary pad) to study localised or systemic immune responses. Tumour and autoimmune models can last maximum up to 100 days. To study systemic immune responses, blood may be withdrawn via tail vain on no more than 5 occasions. Animals may also be monitored for tumour and/or other tissue growth or response using non-invasive imaging under general anaesthesia. Animals will be allowed to fully recover between anaesthetics. Throughout the experiment, animals will be monitored daily for signs of tumour growth or other disease and/or treatment associated symptoms as appropriate. All animals will be humanely culled at the end of the experiment. Following this, tissues (spleen, lymph nodes, vital organs) may be harvested from these animals for analysis by a variety of techniques in the lab.

What are the expected impacts and/or adverse effects for the animals during your project?

Administration of substances: Administration of substances or cells and the withdrawal of body fluids will be will result in no more than transient discomfort and no lasting harm- injections causing momentary needle stick pain. Application of topical substances on the skin can result in scratching behaviour, and therefore if necessary their claws may be clipped to reduce scratching. If the skin becomes broken or infected mice will be treated with Baytril or similar, and/or with an antihistamine after consultation with our named vet. In some experiments the injection of antigen and/or immunomodulatory agent may cause a transient adverse reaction, for example those investigating the effect of a treatment on a secondary response, the induction of long-term memory or those requiring treatment with an immunomodulatory mAb which may induce cytokine release. These effects can occur acutely, within a few minutes, or may not develop for several days and are typically characterised varying degrees of by pilo-erection, hunched posture, lack of responsiveness and shallow breathing; in a proportion of mice (say 1 of a group of 6) the reaction may be more severe, with prostration. To alleviate the symptoms, mice are placed on a warming pad, stimulated, and monitored until they recover and can be returned to their cages; this is usually within 1.5 h-2 h. Symptoms remain within the moderate severity limit of the protocol. Once recovered, the mice do not exhibit any further adverse effects.

Fasting: Fasting per se is unlikely to cause any adverse effects in healthy animals, but may increase the likelihood of an individual animal experiencing an unexpected adverse event following substance administration. However, the overall incidence of such events is still expected to be low (<5%). Animals dosed following fasting will be monitored regularly within the first 3 hours post- dosing. Beyond 3 hours, the frequency of monitoring will be judged on previous experience with the specific compound and dose.

Whole-body imaging: No adverse effects have been noted with previous use of the stated imaging techniques, which incorporate a means of maintaining animal body temperature. The severity of repeated imaging sessions is related to repeated anaesthesia, rather than the imaging itself. Frequency and duration limits set out in the BSU Refined Guidelines for Imaging will be observed. For repeated imaging, no animal will undergo anaesthesia/imaging unless it is judged to have recovered from the previous occasion by the personal licensee, or when necessary in consultation with the NVS and/or the NACWO.

Tumour models: The typical animal is given tumour as a suspension of cells in suitable vehicle (e.g. saline, or, for some subcutaneous injections, mixed in a solution of an inert matrix material such as matrigel). Mammary pad injection (up to 100µl) may be used to inoculate breast cancer models; no adverse effects are expected due to injection site. The tumour is then allowed to grow to a reasonable size (see below) at which point the animal is humanely killed. The overriding consideration for humane endpoints must be the health of the animal. Experiments will be terminated before or at the first signs of, tumour associated symptoms, such as anaemia, laboured respiration, bleeding or other discharge from orifices, abdominal distension or incontinence or diarrhoea lasting more than 48 hours. However, health is also monitored by assessing the overall condition and behaviour of the mice (Foltz and UllmanCullere (1999) Lab Animal, Guidelines for Assessing the Health and Condition of Mice), including facial expression (www.nc3rs.org.uk/assessment-pain-using-facial-expressions-laboratory-mice-rats-rabbitsand-macaques). In all cases, the size of tumour(s)will be the minimum to achieve the scientific objective. Our humane endpoints are based on recognised NCRI guidelines described by Workman et al. [2010] Br J Cancer 102:1555). If an animal has reached the humane end-point of disease or is displaying signs of distress/suffering, the technicians will either cull the animal or inform the personal licensee that the animal should be culled immediately.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Only mice are used for this project. Procedures conducted on all animals are expected to yield mild moderate severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Immune modulating agents act upon multiple cell types across different organs and tissues within the body concurrently and this cannot be adequately modelled in vitro at the current time. Similarly, to study the interactions between an ongoing immune response and a growing tumour, or to evaluate immune related adverse effects, there is unfortunately no viable alternative to in vivo modelling using animals.

Which non-animal alternatives did you consider for use in this project?

We are committed to replacing mice where possible and we evaluate immunotherapeutic agents on cell lines in vitro when we can. We have considered in vitro assays using primary cells and 3D organoid modelling, but they do not accurately reflect the complexity of the tumour microenvironment in a natural setting. However, we will use PBMC and end-organ biopsies from patients treated with immunotherapy who developed adverse effects to study immune cell composition and biomarkers of toxicity.

Why were they not suitable?

Although such in vitro assays may provide information in relation to biomarkers of immune toxicities, and correlative measures of immunotherapy effectiveness and risk of adverse events, they will not shed light on the mechanistic basis of immunotherapy and immune toxicities, where immune cells interact within different organs and tissues. Such interaction cannot be modelled in vitro assays.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For all work, we will adopt the general principles outlined by the Academy of Medical Sciences (October 2015), including adoption of appropriately powered and robust experimental designs, detailed protocols and specific analysis plan for all studies, with expert statistical advice from senior statisticians affiliated to the Centre. Experiments are always designed with the fewest animals consistent with obtaining statistically valid results. We are using inbred strains of mice, so within group variability is also reduced. Replicate experiments will be performed to ensure reproducibility and when appropriate such studies may be combined to increase sample size, and when possible, treatments will be tested with more than one strain and/or tumour.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have where possible, planned to evaluate immunotherapeutic agents on cell lines in vitro and considered using primary cells and 3D organoid modelling. We will archive tissues from experimental animals to avoid experimental repeats and account for the influence of variables and address sources of bias to yield robust and reproducible data, ensuring that the data from every animal is utilised to its full potential. We will reduce bias by random allocation and blinding to ensure researchers analysing experimental outcomes are unaware of the treatment received until the final statistical analysis. We have ensured that experiments are well designed, adequately power and analysed correctly which will all help reduce animal use whilst increasing the scientific validity of the results.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will conduct small scale pilot studies before starting a major experiment to ensure that the experiment is logistically efficient and to give some preliminary indication of likely results. All experiments will be carefully pre-planned, and not changed while the experiment is in progress. We will also statistically analyse the data after the completion of each experiment so that the results will aid in planning future experiments. Measurement error will be minimised by careful technique and good instrumentation, and blinding the researcher to treatment allocation. Where multiple inter-relating parameters are to be evaluated, larger factorial experiments are performed to prevent use of excess mice as controls. In recent years significant technological advances have enabled more information to be obtained from one individual mouse than was previously possible (e.g. using multi-parameter flow cytometry and micro-array technology), enabling multiple parameters to be assessed simultaneously from small samples. These technologies thereby facilitate longitudinal studies and reduce the need to cull multiple mice at different time points to sample from the spleen for instance; we aim to fully exploit these new techniques fully where possible. We will also ensure that any tissues collected during/after animal studies are archived and stored appropriately, avoiding unnecessary repetition of experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The typical animal is given cells, tumour cells or substances as a suspension in a suitable inert vehicle (e.g. saline); no adverse effects are expected due to injection site. The tumour is allowed to grow to a reasonable size at which point the animal is humanely killed. Daily inspections are usually carried out by experienced animal technicians familiar with the models. The overriding consideration for humane endpoints is the health of the animal. Experiments will be terminated before or at the first signs of, tumour associated symptoms, such as anaemia, laboured respiration, bleeding or other discharge from orifices, abdominal distension or incontinence or diarrhoea lasting more than 48 hours. Use of
Imiquimod cream to induce autoimmune inflammation of the skin to model human psoriasis is known to be safe with minimal pain and suffering.

Why can’t you use animals that are less sentient?

Mice are the least sentient mammal species with an immune system similar to humans. Mice represent a relevant animal model for these studies and the clinical successes now being reported using immunomodulatory drugs against cancer were dependent on data arising from such murine studies. Numerous mouse cancers have been studied and the availability of genetically altered strains, and commercially available reagents aids this research.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Environmental enrichment, good husbandry and frequent monitoring ensure high welfare standards. All animal experiments will be conducted in a humane manner, with particular consideration for highest standards of animal welfare as well as for the quality of the science. Any animal experimentation within the institution is fully justified, and death will not be an experimental endpoint in any studies, and animals will be humanely culled as soon as the objective of the experiment has been achieved.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will adhere to the guidance issued by relevant authorities including the
Home Office (https://www.gov.uk/guidance/animal-research-technical-advice),
NC3Rs (https://www.nc3rs.org.uk/guidelines) and the
University of Liverpool (https://www.liverpool.ac.uk/research-integrity/animal-research/ legislationgovernance-and-standards/) and report them in accordance with the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep abreast of literature and guidance from the NC3Rs and Home Office in relation to the framework for performing humane animal research and embed in our studies. We will also work with colleagues to develop viable alternatives such as advanced organoid modelling and computational biology based on latest science and technology as an alternative to animal experiments. We will actively participate in workshops organised by the relevant authorities that provide an update on advances in 3Rs.

Home Office

NON-TECHNICAL SUMMARY

214. Understanding musculoskeletal ageing

Project duration

5 years 0 months
Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs

and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b) **Key words**

bone, cartilage, osteoarthritis, osteoporosis, inflammatory bowel disease

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand the mechanisms underlying musculoskeletal health, and pathological ageing

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Musculoskeletal conditions are at the forefront of ageing-related conditions: the World Health Organisation has described them as “leading causes of morbidity and disability, giving rise to enormous healthcare expenditures and loss of work”. Osteoarthritis is one of the most frequent ageing-related musculoskeletal diseases; the World Health Organisation estimates that 10% of men and 18% of women aged over 60 already have symptomatic osteoarthritis. Further, 40% of women over 50 years will suffer an osteoporotic fracture and 1 in 500 people in the UK are affected by ulcerative colitis with up to 41% of these also affected by associated osteoporosis.

Understanding the mechanisms underlying these conditions will provide invaluable insights and will have broad translational potential. It will, therefore, likely lead to long-term patient benefit and societal impact from a contribution to global economic activity. The clinical benefits are likely still distant but they are potentially huge and their healthcare, financial and societal impact is only set to rise in the ageing population.

What outputs do you think you will see at the end of this project?

The outputs generated from this project will contribute and complement ongoing research into musculoskeletal health. The majority of the UK population will be affected by a bone or joint disease as they age. These may include bone loss and fractures characteristic of osteoporosis, osteoarthritis or even cartilage to bone conversion such as in dyschondroplasia. The proposed studies will therefore provide invaluable new knowledge of the mechanisms which lead to these diseases, and ultimately offer new approaches to treat them. Further, it will generate outputs in the form of: (i) peer reviewed publications (expected >10) (ii) invited seminars (iii) oral and poster presentations by group members at scientific conferences (iv) future grant applications for example to the MRC (v) lay articles for magazines (e.g. The Conversation) (vi) public lectures (e.g. Pint of Science). Together, these will benefit immediately the scientific community. In the long-term this basic research which will be extended and refined by others and may lead to drug targets and treatments for many musculoskeletal diseases.

Who or what will benefit from these outputs, and how?

The immediate beneficiaries of this work will comprise academic researchers in multiple biological disciplines, including, but not limited to bone biology, cartilage biology, osteoporosis, osteoarthritis, rare joint diseases, and joint imaging. This research will, therefore, benefit a wide range of researchers across the globe, with whom future collaborations can potentially be formed. These partnerships will encourage and facilitate interdisciplinary research and all parties would benefit from this. In the long-term, this basic research will be extended and refined by others and may lead to drug targets and treatments of many musculoskeletal diseases which will ultimately benefit members of the public suffering with these debilitating diseases.

How will you look to maximise the outputs of this work?

I always strive to maximise the outputs of my research and will continue to do so for this work. I work closely with national and international colleagues where group efforts are made to understand these biological mechanisms underpinning bone and cartilage disease. I will continue to nurture these productive collaborations for this work, as well as look to foster new ones where appropriate. I am fully committed to ensuring that any novel results generated are disseminated as broadly as possible across biomedical academic communities and that data are available for future interrogation. Similarly, I will always look to publish unsuccessful approaches as I believe these are just as important in our pursuit of understanding, and within the musculoskeletal field, journals have often had special issues focused on these studies. Through the work generated by this project, I will also continue to play an active role in the public understanding of science relating to bone and joint diseases, and methods for prevention/management.

Species and numbers of animals expected to be used

- Mice: 3200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Here we will use mice as the genetic tools and models required for achieving the aims of this project are readily available in this species. Further, mice are required to enable us to examine the 'whole joint' rather than the separate components of the joint. We will be breeding genetically modified mice at all ages, however the protocols we use will be in juvenile, adult and aged mice. This is because we are interested in understanding musculoskeletal health across the life course and in particular, with ageing. It is, therefore, important that we consider our models in the different ages detailed.

Typically, what will be done to an animal used in your project?

Typically, a mouse will undergo induction of a skeletal pathology such as osteoarthritis or inflammatory bowel disease. Before or after this, they may receive an injection of a compound to examine whether this protects against the skeletal pathology. Mice will receive no more than two regulated procedures, and experiments will last no longer than 8 weeks post skeletal induction.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the protocols in this project will not result in any adverse effects. Some mice will have surgery and, therefore, there is a risk of infection, although we will take every precaution to ensure this does not happen. Similarly, there is a small risk of death from anaestheisa given. Those which develop inflammatory bowel disease may experience weight loss, however, we have previously shown that once the treatment is stopped, mice regain this weight.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The highest severity that a mouse will experience in this project is moderate. The majority of animals will experience a mild severity (approx. 60%).

What will happen to animals at the end of this project?

- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this application requires a physiological context, and, therefore, this project will predominantly adopt an *in vivo* approach with mice as the experimental model. Use of mice to provide the 'whole' organ is required to study the objectives so they match as precisely as possible the circumstances in humans. It is also important to note that age-related joint deterioration is a 'whole organ' event; studies that may use models of the joint's constituent

components separately would, therefore, only provide insight into only some aspects of the joint function. Similarly, removing the activity of a gene provides information about what that gene normally does in a physiological (whole body) context and this information cannot be obtained from cell culture models where, for example, cellcell interactions and whole body regulatory pathways are lost. Also, the genetic tools and models required for the studies detailed herein are readily available in this species.

Which non-animal alternatives did you consider for use in this project?

In vitro ("test-tube") and cell culture based alternatives have been, and will be, invaluable to my research and I will always consider them in my experimental design wherever possible. I have fully acknowledged their strengths, reviewed their use for others, but am aware and appreciate their limitations, as detailed below.
Why were they not suitable?

In vitro approaches have a number of recognised limitations. Ultimately, they fall short of providing the integrated, organ-level, physiologically intact environment that animal models provide and, thus, make interpretation of indirect effects of agents on bone impossible to detect. These *in vitro* approaches also fail to produce the range of structural abnormalities in joint architecture that can be seen, and do not represent all *in vivo* tissues.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers are based on my previous work. This was used to estimate the minimum number of rodents required for establishing significant differences between groups.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will always aim to reduce the numbers of animals we use, and routinely use the NC3Rs' Experimental Design Assistant. Power analyses are always applied in order to identify the minimum number of animals that we need to use in order to answer the specific question being posed. For example, we have established that in our surgical model of osteoarthritis (DMM), a minimum of 6 mice per group is required to secure statistically significant differences. Wherever it is possible we will also exploit contra-lateral limbs as controls in order to reduce the numbers of animals required still further.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will always adopt efficient breeding strategies informed on our previous work and knowledge of the mouse strains we are working with. Pilot studies will always be conducted where appropriate. In our studies, we will also

take repeated measures on the same mice where possible, thereby, enabling serial data acquisition and removing the need for the humane killing of multiple groups of mice at set time-points. This will be further advantaged by our current attempts to acquire funding for an *in vivo* computed tomography scanner which allows imaging of bone micro architecture without the need for humane killing. We have many collaborations with other scientists interested in various other related systems and so maximum use of animal tissues is always made.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project will use mouse models. We have chosen mice because their basic skeletal biology is very similar to humans and the models to be used here e.g. joint loading and DMM model, have been developed for, and are widely used in, mice. Furthermore, there is the advantage of readily available gene edited mouse models and reagents for downstream analyses.

Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. Drugs will be administered at non-toxic dosages and if unknown, this will be carefully tested. Our use of surgical approaches will be kept to a minimum and appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia.

Why can’t you use animals that are less sentient?

Here, we will use mice as they are the most suited to be able to answer the biological questions which we pose. The genetic tools and models required for achieving the aims of this project are readily available in this species. Further, mice are required to enable us to examine the 'whole joint' rather than the separate components of the joint, and to take into consideration the whole physiology of the musculoskeletal system which would not be possible in less sentient species such as zebrafish. Species such as zebrafish would also not enable us to understand the effects of loading on the skeleton, and are not easily translatable to humans due to their skeleton developing differently. This project will use juvenile, adult and aged mice because we are interested in understanding
musculoskeletal health across the life course and in particular, with ageing, and hence more immature life stages cannot be used.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have carefully considered each of the procedures described in this application in order to minimise pain and distress experienced by animals, and to enhance their well-being. Indeed, animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. Animals will be handled for one week prior to experiments happening to minimise stress, and anaesthesia and analgesia will be used whenever appropriate and possible. Animals will receive extensive post-operative monitoring and care to ensure minimal welfare costs for the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I already have and will continue to refer to the 'Guidance on the
Operation of the
Animals (Scientific
Procedures) Act 1986' and will speak with our NVS and others to ensure that I am kept up to date with the published best practice guidance. I will also consult published guidance such as *'Laboratory Animal Anaesthesia and Surgery'* Paul Flecknell, and the LASA Guiding principles on good practice.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The Bioresources Users' Group (BUG) regularly meet and welcome both internal and external speakers to discuss current advances in the 3Rs. Regular newsletters from the NC3Rs and LASA are also distributed.

Home Office

NON-TECHNICAL SUMMARY

215. Understanding skeletal disease and pathological soft tissue calcification

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Vascular calcification, Bone formation, Therapy

Animal types	Life stages
Mice	neonate, juvenile, adult
Rats	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to improve our understanding of the processes which cause skeletal and vascular disease. It will also investigate the complications which can arise in bone and blood vessels as a result of diseases in other tissues.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Maintaining bone mass is important for healthy ageing; however, the skeleton does not exist in isolation and its function is influenced by other tissues. Consequently, many common diseases (e.g. chronic kidney disease (CKD or kidney failure), diabetes) are associated with significant skeletal problems (e.g. bone loss). Many of these conditions are also characterised by unwanted and harmful soft tissue calcification (e.g. calcification of the blood vessels which is known as vascular calcification). The processes which lead to the development of these skeletal and vascular problems are not fully understood and lack effective treatments. Therefore it is important to improve our understanding of what causes these issues. Ultimately this knowledge may lead to the development of new drugs to treat these common problems.

What outputs do you think you will see at the end of this project?

The studies performed under this licence will increase understanding of the processes that lead to the development of skeletal problems and harmful calcification of the arteries (known as vascular calcification) in several common diseases (e.g. chronic kidney disease (CKD or kidney failure), diabetes). Ultimately this may lead to the identification or development of compounds that can be used to prevent or treat vascular calcification without exerting negative effects on the skeleton. Experimental outputs will therefore include:

1. Publications/conference presentations describing our research findings. This will provide important new information to other researchers in the field about the processes involved. It may also be interest to industrial partners.

2. Refinement of protocols to reduce animal use.

3. Identification of compounds that warrant further investigation as potential treatments for vascular calcification and/or skeletal problems associated with other diseases.

Who or what will benefit from these outputs, and how?

Many of the conditions which lead to skeletal problems and/or vascular calcification are much more common in older people. Given the ageing population, these problems are expected to increase in prevalence and so finding new therapeutics is essential. Vascular calcification, in particular, does not currently have any effective treatments. This means there is a need for research to understand the processes involved and to identify compounds of interest. Due to the time required for drug development processes, translating basic science findings to clinical benefit is likely to take many years.

In the shorter term, the benefits will primarily be for the broader scientific research community. Data generated will be published in a timely manner to ensure effective dissemination to the skeletal and vascular biology fields, as appropriate. Any refinements in protocols was also be shared to ensure that improvements in methodology can be more widely adopted.

How will you look to maximise the outputs of this work?

Findings will be presented at appropriate national and international conferences to ensure rapid dissemination of new knowledge, protocols and refinements to a broader scientific audience. Data will also be shared with new and established collaborators within the field to inform and refine future studies of a similar nature. Once individual projects are complete the results will be written up in a timely manner for publication in a peer-reviewed journal.

To prevent unnecessary repetition of *in vivo* studies we will aim to publish all findings, both positive and negative. Publication of research findings is also disseminated to a general audience via the institution website.

Species and numbers of animals expected to be used

- Mice: 3,600
- Rats: 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Protocols 1-3: The ability to switch genes on or off in rodents (mice and rats) has yielded a lot of important information about how tissues work. Study of these animals provides an important research tool that helps to increase our understanding of how diseases develop. There are a number of diseases (e.g. chronic kidney disease (CKD or kidney failure), diabetes) which cause problems in the skeleton and also lead to the development of harmful calcification in the arteries (known as vascular calcification). In some cases, animals will be fed a high fat to mimic a western diet. Using these genetically altered animals in this project will help us to understand the processes which lead to the development of these unwanted effects. Furthermore, studying animals at different life stages provides important information on the impact of ageing on these processes.

Protocols 4 and 5: In order to determine whether potential treatments can prevent disease-induced vascular calcification and skeletal problems it is necessary to use animals with that disease (e.g. CKD). These experiments are performed on adult rodents as the diseases being modelling are typically associated with ageing.

Typically, what will be done to an animal used in your project?

Protocols 1-3: Here, animals with genetic changes will be bred for experimental purposes. Animals will either be used to maintain breeding colonies, to isolate cells or tissues or transferred for use in protocols 4 or 5.
Protocol 4: Here, animals will be fed a modified diet which results in the development of chronic kidney disease (CKD) and the associated skeletal and vascular problems. Animals will be fed this special diet for the duration of the study (up to 12 weeks). During this time, animals will have regular blood tests and be given compounds which could prevent the unwanted consequences of the disease. At the end of the study, all animals will be euthanised and tissues collected for experimental analysis.
Protocol 5: Here, animals will be fed a modified diet which results in the development of the skeletal and vascular problems associated with ageing. Animals will be fed this special diet for the duration of the study (up to 12 weeks). During this time, animals will have regular blood tests and be given compounds which could prevent the unwanted consequences of ageing. At the end of the study, all animals will be euthanised and tissues collected for experimental analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

Protocol 1: The genetic alterations in these animals are not expected to cause any significant adverse effects.
Protocol 2: The genetic alterations in these animals may lead to the development of disease and the associated symptoms. In most cases these effects will worsen with age.
Protocol 3: The genetic alterations in these animals and the feeding of a high fat diet are not expected to cause any significant adverse effects.
Protocol 4: Animals on the modified diet in this protocol will develop kidney failure and the associated adverse

effects which include reduced food intake and weight loss. Since this model reliably mimics the human clinical symptoms of kidney failure adverse effects are likely in all animals. Symptoms are expected to appear within 1-2 weeks of study initiation and will gradually worsen over time; after onset, adverse effects will last until the study ends. Animals will be carefully monitored throughout the study and if symptoms are approaching the limit of severity they will be euthanised via schedule 1 methods and tissues collected for analysis.
Protocol 5: Animals on the modified diet in this protocol are not expected to experience any adverse effects with the exception of transient weight loss.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Protocol 1: The expected severity of this protocol is mild. It is anticipated that all animals will experience this level or a sub-threshold severity.
Protocol 2: The expected severity of this protocol is moderate. It is anticipated that all animals will experience this level or a mild severity.
Protocol 3: The expected severity of this protocol is mild. It is anticipated that all animals will experience this level severity.
Protocol 4: The expected severity of this protocol is moderate. It is anticipated that all animals will experience this severity.
Protocol 5: The expected severity of this protocol is mild. It is anticipated that all animals will experience this severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Within the body, there are numerous interactions between the different tissues. As a result a disease in one particular organ/tissue can cause problems in another. This project will investigate how diseases such as chronic kidney disease (i.e. kidney failure) and diabetes cause skeletal problems and harmful calcification of the arteries (vascular calcification). To learn more about these conditions and to find effective therapeutic treatments requires a number of experimental approaches.
In vitro (i.e. in the lab) work using cells obtained from animals can provide important information about how things work. Particularly useful in improving our understanding are cells that are isolated from rodents that have had genes switched on or off. However, one limitation of *in vitro* studies is that they cannot replicate the 3D structure of tissues or model the interactions between different tissues. Therefore, *in vitro* work is most informative when used in combination with whole animal (or *in vivo*) studies. This project will use both methods to address our research objectives.

Which non-animal alternatives did you consider for use in this project?

Where possible we use human vascular cells for lab experiments but human bone cells are very difficult to obtain. Therefore bone cells for study need to be isolated from rodents. At present there are no effective non-animal models that allow the skeletal problems or vascular calcification associated with disease to be studied in the same system.

Why were they not suitable?

Non-animal systems that can reproduce the interactions between bone, blood vessels and other tissues do not exist. Therefore, at present there are no alternatives to whole animal models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimated numbers are based on over 10 years of animal work. Extensive experience in bone and vascular research has provided a detailed understanding about the number of animals required for the different experimental approaches employed. It also takes into account typical inheritance patterns when breeding genetically altered animals and intragroup variability for whole animal *in vivo* work.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The techniques employed in our research are continually being refined to maximise the experimental outputs from the animals used. These changes have been used to inform the experimental design in this project licence. For example, recent refinement of isolation methods means that four distinct cell types can now be obtained from a single animal; this represents a significant reduction in the number of animals needed for *in vitro* work. In addition, improvements in the methods used for *in vivo* studies has reduced the degree of variability between animals. This means that overall the number of animals needed in each experimental group can be reduced.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

As mentioned above we have designed our protocols so that four distinct cell types can be isolated from a single animal or group of animals. This is particularly useful when isolating cells from genetically altered animals which may only be available in limited numbers. Furthermore, breeding programs will be designed to limit the generation of animal s which cannot be used for experimental purposes.
Where necessary, pilot studies will be used to test poorly characterised compounds prior to commencing a full study. This will ensure that excess animals do not have to experience or be euthanised due to unforeseen adverse effects.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques

during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Protocols 1 and 2: This work will involve the breeding and maintaining of rodents (many with genetic changes) for research purposes. The majority of these animals are not expected to experience any significant pain or distress.

Protocol 3: This will involve feeding certain animals a high fat diet. This is not expected to have a significant impact on welfare.
Protocol 4: Chronic kidney disease and the associated skeletal and vascular problems can be induced in rodent models via the diet or by surgery. We have opted to use modifications in diet because this method is less invasive, shorter in duration and is more reproducible.
Protocol 5: This method uses a modified diet to allow the study of the vascular and skeletal problems that can develop as a consequence of ageing. This approach has been refined to minimise the adverse effects and reduces the need to use aged animals.
Animal suffering will be limited in all our studies by our strict monitoring of actual severity and severity limits. Our protocols are also designed not to produce excessive trauma or suffering. In all cases, animals will be euthanised if they approach the limit of severity.

Why can't you use animals that are less sentient?

The conditions being investigated are most often associated with ageing and cannot be sufficiently reproduced in animals at an immature life stage. Furthermore, since bone loss and the development vascular calcification take a number of weeks to occur it is not possible to carry out these protocols on terminally anaesthetised animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Protocols 1-3: All animals will be subjected to regular monitoring to ensure welfare is maintained. If an animal model is new or poorly characterised, levels of monitoring will be increased until the model is characterised.
Protocols 4 and 5: Animals purchased specifically for a study will have a 1-2 week acclimatisation period prior to work starting. Animals will undergo a health check twice a week unless part of a pilot study testing a poorly characterised compound. These animals will be subject to enhanced levels of monitoring. Options for using creatinine levels to refine protocol 4 will also be explored.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All whole animal *in vivo* studies will follow the ARRIVE guidelines. Administration of compounds will follow the LASA guidelines. Power calculations will be performed prior to every study to confirm that enough animals are included to ensure statistically relevant results.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Advancing the 3Rs and ensuring animal welfare are central to our research ethos. Attendance at internal seminars and training courses aimed at promoting and improving best practice as well as external seminars and relevant conferences will ensure that the PPL holder and any PIL holders working under this licence are kept up to date with relevant new developments. Regular contact with international collaborators using similar whole animal

models will ensure that any refinements developed in other research institutions can be quickly incorporated to the studies performed under this licence (subject to appropriate PPL amendments).

Home Office

NON-TECHNICAL SUMMARY

216. Understanding the biology of epithelial cancers

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cancer, Therapy, Preclinical Modelling

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Epithelial cancers such as bowel, liver or pancreatic cancer account for the majority of diagnosed cases, and commonly respond poorly to treatment. To develop better, treatments which are likely to have a positive effect in patients we need to better understand a number of features of how cancers behave. These include understanding how they form, how they grow and they progress to more dangerous disease. We also need to understand how

cancers differ from normal tissue, and what makes response to any treatment effective. To understand this process in its entirety, our study aims to develop animal models which resemble human cancer as closely as possible, then uses these to understand how cancer develop and grow, and use this to identify key new potential treatments.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Epithelial cancers represent up to 85% of all cancer diagnoses in the UK presently. They also represent a pressing need for new treatments, with little progress in terms of development of new targeted approaches seen over the last 20 years. Using bowel cancer as an example, it is second most common cause of cancer related death in the UK, accounting for 10% of all cancer deaths annually. Patients with late-stage, metastatic disease fare worst, with a 5-year survival rates following diagnosis at stage 4 of around 10%. In this patient group, while surgery is the most effective means of increasing patient survival, only ~15% of patients with metastatic disease are suitable for this. Moreover, given that classical chemotherapies and targeted approaches are often ineffective in bowel cancer, there is a real need to identification and validate new potential targets which might be effective as a treatment and might benefit patients. Key to this process is the generation of animal models which accurately reflect human disease, increasing the likelihood of developing successful treatments.

What outputs do you think you will see at the end of this project?

This work will advance our knowledge of the cellular processes which govern tumour initiation, progression and therapeutic resistance in a setting which is directly translatable to defined patient populations. Ultimately, this work will identify new actionable targets for therapy in epithelial cancer. We will seek to publish all experimental data that arises from this work in order to maximise benefits arising from animal usage.

Who or what will benefit from these outputs, and how?

The identification of novel targets, pathways or process which are relevant to cancer progression and therapeutic resistance in cancer will ultimately lead to patient benefit in the longer term. In the medium term, the identification and validation of changes which are common to specific subtypes cancer, may be a valuable way to identify patients who either will or will not respond to any given treatment.

How will you look to maximise the outputs of this work?

We currently participate in number of large collaborative networks aimed at dissemination of data generated from *in vivo* models of cancer, alongside integration of data generated from these models, as well as human clinical samples and advanced culture systems. The ultimate aim of these collaborative approaches is to make high quality, robust and impactful data available to the wider research community, drive appropriate translation of findings into the clinic, and ultimately benefit patients. We aim to disseminate findings from all research carried out as part of this project through seminar, symposium and conference presentations, both within our establishment and to the wider cancer research community, alongside publication of multiple peer-reviewed manuscripts.

Species and numbers of animals expected to be used

- Mice: 180,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse models described in this project are genetically modified so that they are susceptible to cancer development. We now have a great deal of understanding of the genetics of both mice and human cancer, and

can use this knowledge to design excellent models. Each mouse model is very carefully designed so that adult mice will develop tumours which very closely resemble those seen in human cancer, and go through all of the stages of development seen in patients. We need to use these models systems because human cancer is an incredibly complex disease - tumours are made up not only from tumour cells, but also immune cells, supporting cells and blood vessels, many of which they rely upon for growth, and which can be important in success or failure of treatments. As there are currently no non-animal models which can reproduce these complex tumours accurately, the development of effective and accurate animal models of cancer is vital for the development of effective treatments for patients.

Typically, what will be done to an animal used in your project?

Around 25% of mice produced under this protocol will have a genetic predisposition to develop cancer. Subsequent genetic modification of these experimental mice will be induced using an injectable agent, typically on one occasion only. A proportion of these (50% of experimental mice, 12.5% of the total) will go on to develop cancer as adults and will be humanely killed when they show symptoms of cancer development without the need for additional procedures. In a small number of cases (<5% of experimental mice), in order to study particular key aspects of human disease, including the process of metastasis, surgical techniques may be used to implant tumour cells grown in the laboratory into organs affected by metastatic disease, such as the liver or the colon.

Approximately 50% of cancer-bearing experimental mice (12.5% of total) will be used in preclinical trials, in which they will be administered with treatments designed to potentially benefit human patients.
These treatments can include drugs, which can be administered orally or by injection, or can include radiotherapy using a specially designed small animal radiotherapy machine. In a small number of cases (<5% of experimental mice, <1% of total), modification of the diet of the mouse might be used to better understand the impact of the treatment, or to enhance its effect. Similarly, in a small number of cases (<5% of experimental mice, <1% of total), tumour growth and response to treatment may also be monitored using advanced imaging techniques which are commonly used for human patients in the clinic, such as MRI, ultrasound or CT scanning. This will require mice to be anaesthetised for short periods of time while images are collected.

All experimental animals will be humanely killed and tissue specimens collected for analysis to maximise the data available from every study.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of mice in this project (75%) will have a subthreshold severity, meaning that they will show no harm, and will undergo no procedures other than earmarking for identification purposes. About a quarter of animals (25%) will have a predisposition to develop cancer, of which a proportion
(approximately 5%) will exhibit no more than mild adverse effects, as they will be sampled at timepoints prior to manifestation of clinical symptoms of ill-health. The remaining animals (20%) will be expected to develop moderate adverse effects associated with tumour development. With respect to procedural burden (e.g. imaging, therapeutic intervention, metabolic labelling), around 20% of animals will experience one or more procedures, which will never exceed a moderate severity.

Under this project, cancer-related genetic changes will be targeted to a tissue of interest, such as the intestine, pancreas, liver or skin, which will result in a specific predisposition to cancer development in that tissue. These animals will then be continually monitored for clinical signs which may indicate tumour growth. These signs can include anaemia, weight loss, swelling of the abdomen and development of palpable or visible tumours. Highly trained staff will monitor for these symptoms, and they are observed to interfere with normal behaviour, reach the limits allowed in the guidelines or have any consequences that are out with the guidelines, mice will be humanely culled and tissues harvested for analysis. Typically mice might exhibit mild clinical signs, which do not impact normal behaviour for around 1-2 weeks, and moderate clinical signs for 24-48 hours before they are humanely killed. Animals which undergo surgical implantation of tumour cells under anaesthetic, may experience transient discomfort (~48hrs), which will be alleviated through administration of pain relief.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All the animals used in this project will be mice, with the following expected severities -

- 70% mild
- 30% moderate

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Cancer is a very complex disease that involves a number of different cell types, including epithelial cells, immune cells, stromal cells and blood vessels. To date, no *in vitro* system has been developed which can faithfully recapitulate all aspects of this complex nature of a tumour *in vivo*. Moreover, our own recent research in colorectal and pancreatic cancers have demonstrated that the immune and stromal components of genetically engineered mouse of models of cancer both align very closely to those of human cancers, and respond to therapeutic challenge in clinically relevant manner. Similarly emerging research in human clinical samples using single-cell sequencing has demonstrated that many of the characteristics of individual immune cell populations in human tumours are very closely resembled by the populations present in mouse tumours.

Which non-animal alternatives did you consider for use in this project?

In undertaking the research outlined in this project we will make extensive use of advanced organoid culture systems to further our understanding of the genetic and/or epithelial determinants of cancer development and progression. This will include the use of human and murine tumour derived culture systems, and will be highly informative with respect to target discovery and validation. These approaches currently represent the best available alternative to *in vivo* modelling in cancer, and will allow us to replace *in vivo* experiments in a number of circumstances. Indeed, in order to further develop these models, and to generate more accurate *in vitro* models, we are actively developing our organoid culture approaches through co-culture with immune and stromal cell populations.

Why were they not suitable?

While organoid culture methods will be used extensively in our research, they are not capable of recapitulating all aspects of complex tumour biology observed *in vivo.* Critically, many of the features of tumour biology which are lacking in organoid culture systems, such as immune evasion and stromal interactions, are now known to be key features in driving both poor prognosis and resistance to treatment in the clinic.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have generated a suite of highly effective complex genetic models of epithelial cancers, which we are now able to position in relation to human disease. Studies carried out with these model systems to date have been highly effective, providing a strong platform of preclinical data on which to base upcoming clinical trials. A combination of knowledge of the complex genetic nature of these model systems, the animal use which was required to generate these models over the previous 5 years, and the further proposed studies outlined in this application have been used to estimate animal use over the period of this licence.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have used statistical approaches to ensure that we use the minimum number of mice to generate significant results. The majority of mice will be generated in a pure inbred C57BL6/J background, which reduces variation between animals and in turn the number of mice required for cohort studies. This allows us to directly compare controls between different experiments and so we can be precise in the estimation of mouse numbers we need for each experiment. In addition, we can consult with experts in the field. At the end of each experiment, data is compared to previous studies by appropriate statistical methods to reduce mouse numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will seek to minimise animal usage through production of mouse colonies using efficient breeding strategies, often through maintenance of stock animals which carry multiple genetic alterations. Where possible, we will reduce generation of control groups through use of inbred mouse strains, and comparison to historic data from previous experimental cohorts. Where possible, pilot studies will be undertaken to allow for power calculation and subsequent estimation of effect size and appropriate group size.

We have undertaken significant effort over the 5-10 years generating new “ex-vivo” models of intestinal cancers which are grown in the laboratory, in the expectation that adoption of these will allow further reduction in animal use. They allow us to tests efficacy of targets “ex-vivo” and so reduce the number of mice we test with genetic knockout or treat with inhibitors “in-vivo”.

Alongside the generation of organoid cultures, we have also developed approaches over recent years which allow us to orthotopically transplant these lines into syngeneic recipients (intracolonic or intrasplenic), which further reduces the number of mice we would need to breed through complex genetic crosses.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Throughout these studies we will use genetically modified mice. By introducing the same mutations found in human cancer to these mice, we can study the development of cancers that accurately resemble human disease. All animals receive the highest standard of care, and will be provided with environmental and behavioural enrichment.

Where possible we will use targeted genetic modification, which allows introduction of cancer driving mutations to specific tissues in adult mice. This approach will reduce any unwanted adverse effects which might result from untargeted genetic alteration. We will use advanced imaging approaches, similar to those used in patients in the clinic, including ultrasound, endoscopy, and PET/MRI to detect tumours at the earliest point possible. Close monitoring of tumour development (using well documented clinical signs and/or imaging) by highly trained staff will ensure any animal suffering is minimised.

Why can’t you use animals that are less sentient?

Cancer in humans is a very complex disease that involves a number of different cell types, including epithelial cells, immune cells, stromal cells and blood vessels. The use of genetic altered mice to study cancer development allows us to understand this process in a mammalian model which has an intact immune system, and develops cancers which very closely resemble those found in patients.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Robust local standard operating procedures for animal care, monitoring and minimal handling are in place, with enhanced social, environmental and behavioural enrichment provided. Lab members will ensure that all animals receive the highest standard of care, with close monitoring on tumour development (using well documented clinical signs and/or imaging) to ensure animal suffering is kept to a minimum. Where appropriate, for example pre- or post- any invasive or surgical procedures, anaesthesia, analgesia, access to palatable food treats and increased monitored will be implemented.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

For all studies we will adhere to published current best practice, and local guidelines. This includes following published guidance related to undertaking ageing studies in mice (Whitehead et al 2014, Wilkinson et al 2019), or *in vivo* studies of cancer in mice (Workman et al 2010). All experiments will be carried out, and any data published, in adherence to the ARRIVE guidelines (NC3Rs, Kilkenny et al 2010).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will consult the NC3Rs guidelines and monitor refinement where such practices are published (NC3Rs website and elsewhere). In our facility, compulsory monthly user forums are also in place for all personal licence holders, and 3Rs advances are introduced here and implemented across the facility.

Home Office

NON-TECHNICAL SUMMARY

217. Understanding the genetic basis of hearing and inner ear disorders

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aims of this project are to use genetically modified mice to explore i) the mechanisms of hearing and

vestibular function, ii) the genetic causes of hearing loss and iii) the signals that may lead to the regeneration of new hair cells in the damaged inner ear

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our senses of hearing and balance depends on specialized sensory 'hair' cells located inside the inner ear. The hair cells are only produced during embryonic life. Their disappearance in adults is the main cause of hearing loss, in particular in the ageing population or after exposure to excessive sound levels. There are also many genetic forms of deafness, which vary in their severity and progression over life. Hearing loss affects more than 10 million people in the UK alone, and the World Health

Organisation estimates 280 million people worldwide suffer disabling hearing impairment. The current treatments for hearing loss are limited to hearing aids or cochlear implants and there is a considerable need for new and improved therapies.

Our understanding of the mechanisms of hearing and deafness has progressed dramatically thanks to genetic studies performed in the laboratory mouse over the past two decades. In the mouse, it is possible to inactivate a specific gene and find out how it affects for example the formation of the inner ear or hearing. In most cases, a gene important for hearing in the mouse turns out to have an equally important role in humans, which has led to progress in the diagnosis and prognosis of genetic forms of deafness. Furthermore, studies in genetically modified mice and recent advances in DNA sequencing technologies are now allowing researchers to identify the genes essential for hair cell formation. This knowledge could open up new venues for therapies: understanding how hair cells form is the first step towards stimulating their regeneration in a damaged inner ear.

In this project, we will use genetically modified mice to explore i) the mechanisms of hearing and vestibular function, ii) the genetic causes of hearing loss and iii) the signals that may lead to the regeneration of new hair cells in the damaged inner ear.

Our work will contribute to a better understanding of the genetic causes of hearing loss and balance disorders. This knowledge will improve our ability to diagnose precisely and predict the progression of these disabilities. By identifying some of the genes essential for hair cell formation and regeneration, it could also help developing new and better therapies for inner ear disorders.

What outputs do you think you will see at the end of this project?

We expect to identify and understand the roles of genes important for inner ear development, function and maintenance. We will also develop new animals models and experimental methods that could be used by other researchers.

Who or what will benefit from these outputs, and how?

In the short-term, the main beneficiaries of this work will be the scientific community at large and the pharmaceutical and biotechnological sectors who will have direct access to our results after publication. Discovering new genes important for hearing could improve the diagnostic of genetic forms of hearing loss, benefiting directly hearing loss sufferers. In the long-term, a better knowledge of the mechanisms of development of the inner ear has also the potential to lead to new gene and pharmacological therapies for hearing loss and vestibular disorders.

How will you look to maximise the outputs of this work?

Our findings will be published in open-access journals to maximize their diffusion. Our groups regularly attend international and national conferences to present their results and have close links to clinicians and charities working directly with hearing loss sufferers. We also participate to a number of outreach events to make the results of our research accessible to the wider public. Finally, we have an extensive network of collaborators in academia and the biomedical sector that will help us to maximise the scientific, clinical and industrial outputs and applications

of this work.

Species and numbers of animals expected to be used

- Mice: 4100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Our understanding of the mechanisms of hearing and deafness has progressed dramatically thanks to genetic studies performed in the laboratory mouse over the past two decades. In the mouse, it is possible to inactivate a specific gene and find out how it affects for example the formation of the inner ear or hearing. In most cases, a gene important for hearing in the mouse turns out to have an equally important role in humans, which has led to progress in the diagnosis and prognosis of genetic forms of deafness. Furthermore, studies in genetically modified mice and recent advances in DNA sequencing technologies are now allowing researchers to identify the genes essential for hair cell formation. This knowledge could open up new venues for therapies: understanding how hair cells form is the first step towards stimulating their regeneration in a damaged inner ear.

Typically, what will be done to an animal used in your project?

We will breed and maintain different lines of mice carrying either specific mutations or genetic alterations known to result in inner ear impairment (causing hearing or balance disorders), or mice with genetic modifications that are not harmful but facilitate the study of a given inner ear cell type.
Some of the animals will be kept for up to 2 years to study their age-related hearing loss and balance function during ageing. In some experiments, we will inject the mice or supplement their diet with Tamoxifen, a compound that can be used to trigger a specific genetic alteration (e.g. deletion of a gene of interest) at a controlled time point. In other experiments, we will need to inject pregnant mice with mitotic tracers to study the potential impact of a given genetic alteration on cell proliferation in the developing inner ear of their embryos.

What are the expected impacts and/or adverse effects for the animals during your project?

Many of the mutant and genetically modified animals to be bred and maintained show no obvious ill effects in the long term, but these animals will be mildly discomforted by the ear clipping procedure necessary for their identification and genotyping.
In our current lines, the ‘breeders’ in which only one copy of the gene has been affected (heterozygous) are normal and do not experience any particular discomfort. However, some of their offspring’s will have both copies of the gene affected (homozygous) and could develop a severe hearing and/or balance impairment, causing moderate discomfort, as well as other pathologies that vary in their severity and tend to worsen with age. The animals developing such side effects will be culled humanely as early as possible to avoid any unnecessary suffering.
For our studies into the mechanisms of development or the inner ear, homozygous animals are usually culled at embryonic stages or soon after birth, minimizing therefore the level and duration of any discomfort or suffering. Tissue collected from these animals is typically processed for genetic and histological analyses, or alternatively maintained in culture for further experiments.
For the other studies aiming at characterizing auditory function in an adult animal, some homozygous mice may be kept until adult stages. The onset of hearing typically occurs at 2 weeks of age in mice, and animals in which the genetic modification can produce harmful side-effects will be carefully monitored and kept for the absolute minimum amount of time required to assess their hearing or balance function.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect that the majority of the animals (90%) will not experience any discomfort or no discomfort beyond the hearing loss or balance disorders associated to inner ear dysfunction or the transient stress associated to the necessary injection/marking procedures.
A small proportion of animals maintained for ageing studies may develop additional pathologies, such as kidney dysfunction or the spontaneous appearance of tumours, causing potentially moderate harm (10%).

What will happen to animals at the end of this project?

- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Understanding the contribution of a specific gene to inner ear development, function, or maintenance necessitates the use of animals since a fully functioning inner ear cannot be produced in a dish, outside of an animal.

Which non-animal alternatives did you consider for use in this project?

Some alternative now exist to produce inner ear sensory cells in a dish, using in particular embryonic stem cells.

Why were they not suitable?

The inner ear 'organoids' derived from stem cells do not allow the testing of auditory or vestibular function or the study of age-related hearing loss. Furthermore, they do not recapitulate fully the normal development of the inner ear, limiting therefore the type of developmental studies that we can perform in this system.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We will use mice carrying either specific mutations or genetic alterations known to result in hearing or balance impairment, or animals with genetic modifications that are not harmful but facilitate the study of a given inner ear cell type. In the course of this 5 year project, we plan to breed and maintain approximately 4100 genetically modified mice. This number is based on our previous experience and that of our collaborators working with genetically modified mice.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Careful considerations were given to the Arrive guidelines and information available through the NC3Rs website using features such as Experimental Design Assistant to ensure that the minimum number of animals are used in this project, while keeping in mind the need to generate robust and reproducible data and providing the best possible welfare for the animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

When possible, each animal will be used for multiple analyses post-mortem. We will use wild-type animals from the transgenic colonies as controls to minimize animal outsourcing.
We also expect that our current work with alternative models and experimental techniques (in vitro stem cell systems) will contribute to a reduction of our use of genetically-modified mice in the course of this project.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The mouse has become the preferred model for many studies of the cellular and molecular biology of the auditory system: there are numerous mutant and transgenic strains available and a high conservation of the genes controlling inner ear development and function in mouse and humans. In choosing the genetic models for our studies, we will use when possible conditional and inducible alleles which are less likely to result in harmful defects. For studies involving a genetic modification that may cause early lethality, animals will be monitored daily and culled as early as possible and before any signs of severe suffering.
Why can’t you use animals that are less sentient?

Studying the function of the auditory and vestibular system necessitates animals that have reached an age when the inner ear is fully developed. Likewise, investigating the effects of specific genetic conditions on ageing and maintenance of the auditory system would not be possible without using aged animals. We are using chicken embryos to study the roles of genes that participate to inner ear development, but it is often necessary to validate our findings in a mammalian model such as the mouse to ensure that the function of a given gene is conserved across species.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In all protocols relying on the administration of drugs, we will perform pilot studies to establish the minimal dose required and the least stressful mode of administration to minimize animal suffering. The animals maintained for ageing studies will be monitored more regularly after 12 months, and we will choose transgenic models (tissue-specific, inducible or not) and breeding protocols that have the least possible impact on animal welfare.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will consult NC3Rs resources, the ARRive guidelines and specific literature about the maintenance of ageing animals and the use of genetically modified mice (accessed online or provided by collaborators) for refining the design and conduct of our experiments.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly consult the information provided by the NC3Rs (website, newsletter, website) and will be kept informed about the latest advances in the use of genetically modified mice and their alternatives by attending specialist conferences and accessing available resources on this topic (website, publications).

Home Office

NON-TECHNICAL SUMMARY

218. Understanding the interplay between different B cell subsets and the gut in immunodependent diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Arthritis, Autoimmunity, B cells, The gut, Bacteria

Animal types	Life stages
Mice	adult, pregnant, embryo, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how the gut-microbiota and gut "microenvironment" influence the differentiation of B cells, in

particular of a subset of B cells called regulatory B cells that can control the development of autoimmunity, as well as to understand more widely how the gut and bacteria contribute to the causes and severity of autoimmunity.
A retrospective assessment of these aims will be due by 12 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This work is important because it has the potential to improve the management of disease in patients with rheumatoid arthritis and systemic lupus erythematosus, firstly by obtaining a greater understanding of the causes and development of these complex diseases, and secondly by providing new microbiota or gut-targeted therapeutic strategies for the treatment of these diseases.

What outputs do you think you will see at the end of this project?

The primary outputs of this project will be publications relating novel basic findings about the outcome of the interaction between gut-bacteria, B cells activation and arthritis. We expect to provide new information on firstly how regulatory B cells develop and how the gut, and the gut bacteria composition influence their development and function in healthy mice, and secondly how changes in gut-bacteria composition observed during the development of arthritis and consequent inflammation in the gut affects their differentiation and the severity of arthritis. In the longer term, we hope our research will have an impact on the way patients suffering from autoimmune conditions are treated. For example, our work will investigate how maintaining gut health (making sure the gut doesn't leak contents into the body) affects the severity of arthritis. The drugs we will use to maintain gut health (either by preventing it from becoming leaky, or by preventing the recirculation of inflamed cells to and from the gut) are currently being tested for their efficacy in gut related diseases and could be repurposed for the treatment of arthritis. Thus, our outputs may include novel treatment strategies.

Who or what will benefit from these outputs, and how?

Academic beneficiaries: Given that the work in our proposed project is looking at basic molecular and immunological interactions of a type of anti-inflammatory white blood cell called regulatory B cells (Bregs), and the potential genes and gut-derived metabolites regulating these interactions in the context of autoimmune disease, the main short-term beneficiaries of our work will be researchers working in the field of immunology, mucosal immunology, rheumatology and autoimmunity.
Industry beneficiaries: As the proposed research is to be carried out in animal models there will be no initial commercially exploitable results. However our results will be used to inform work developing therapies for patients (e.g. cellular therapy using Bregs, or manipulation of the metabolites in the gut to alter arthritis symptoms) that may, at a later point, result in commercially exploitable findings.

Non-academic beneficiaries: In the longer term, we hope our research will have an impact on the way patients suffering from autoimmune conditions are treated. If our hypotheses are confirmed by the experiments proposed under this licence we will be able to transfer this knowledge into patients with autoimmunity. Bregs are vital in restraining excessive inflammation and in regulating immune responses including those developing in autoimmunity and in cancer. Thus, for example, our extensive profiling of murine Bregs may identify molecules or genes that can be used to better define Bregs in both mice and humans. These markers they could then be used to better identify patients with abnormally low, or high, Breg numbers, possibly stratifying patients into correct treatment groups.
Similarly, strategies to enhance butyrate production (butyrate is a metabolite produced by gut bacteria that has been shown to reduce symptoms of arthritis in mice, partially by enhancing Breg function) by gut bacteria may be a non-invasive way of aiding tolerance in patients with inflammatory disorders. Manipulation of gut bacteria directly (via administration of antibiotics or probiotics) may lead to an enrichment of Bregs over pathogenic B cells. In

addition, other biological therapies aimed at restoring potential gut-barrier disruption or recirculation of gut-resident inflammatory cells into the joints could also explored in clinical trials. We anticipate that we need a maximum of 4 years before we are ready to translate some of our results to the clinic.

How will you look to maximise the outputs of this work?

In order to inform these beneficiaries of our work we will present our data at local departmental and divisional meetings as well at national and international meetings, in particular at meetings such as the British Society for Immunology, the British Society for Rheumatology, the European Congress of Immunology and the American College of Rheumatology and Keystone Symposia. We have the opportunity to present our findings at least 2-3 times a year at internal meetings, and we hope to present our data at least once a year at international meetings from the second year of the proposed work. Once completed, we intend to publish our data as open access articles in the highest possible impact journals, so that our findings are available to the widest possible audience, and make our data available on widely used public repositories such as GEO, while programming codes will be published via GitHub.

Species and numbers of animals expected to be used

- Mice: 8500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Unfortunately, at present we are unable to model systemic diseases such as arthritis *in vitro*, as autoimmunity affects multiple organs via complex crosstalk between different cell types. Answers that are more representative of human disease are obtained when we study directly the immunobiology of kidneys, liver, spleen, blood, lymph nodes and joints in diseased mice. This allows us to gain an overview of the potential of therapeutics. Presently we cannot do this either *in vitro* or *in silico*.
Mice and humans are genetically and physiologically very similar. The protein coding regions of the two genomes are on average 85% identical, and as much as 99% for some genes. In terms of the study of autoimmunity, this means that mouse models of disease have similar physiological characteristics and clinical symptoms to their human counterparts. As such, using mice as a model system for human autoimmunity has been proven to be very useful in research.
There is a wealth of experience using mice to measure immune responses. The availability of well described inbred, congenic and genetically altered strains, as well as well described spontaneous and inducible models of autoimmunity makes them currently the most appropriate species in which to carry out this work. In addition, it is now straightforward and affordable to generate genetically altered mice to allow the precise dissection of the contribution of individual molecules to the immune system and disease. Thus, mice provide an excellent experimentally tractable system to investigate autoimmune disease development, immune responses, and therapies.

Typically, what will be done to an animal used in your project?

Typically, a mouse used in our project will be induced to have a type of experimental arthritis. We will then compare the effect of different treatments, or the effect of changing the gut bacteria, on symptoms of arthritis and the activation of the immune system. The mice we use will typically be 7-8 weeks old, with fully adult immune systems, and the duration of experiments will typically be 2 to 3 weeks from first treatment to culling.

To induce arthritis, we typically first give a fully anesthetised (using inhaled anaesthetic) 7-8 week old mouse an injection of the molecule that the immune response is to be directed against to cause arthritis. This molecule will be in emulsion with an oil-based adjuvant, which is a substance that causes the mouse's immune system to recognise that molecule as dangerous, and to become specifically active against it. This injection is in a small

volume (0.05-0.1 ml) and is given just under the skin, in the fatty tissue on the rear of the mouse near the base of the tail. The mouse is then allowed to wake up and is returned to its housing. The mice will be unconscious for less than 5 minutes. This injection can cause some irritation, like a strong reaction to a BCG immunisation, for the first few days until the adjuvant is absorbed.
One week later we will anaesthetise the mouse again and, using a very fine needle, we will inject the same molecule in a very small volume of saline like solution (0.01 ml) directly into one knee. In the other knee we will inject the same volume of saline like solution with no molecule so that we can compare an arthritic knee to a healthy knee in the same mouse. Again, the mice will be unconscious for less than 5 minutes. These injections will cause some discomfort, however the mice are able to walk and feed as soon as they wake up and are returned to the cage.
Over the course of the next week mice will develop swelling in the knee that was injected with the molecule. This swelling starts on the first day and reaches a peak by 3 days, after which the swelling subsides and usually completely disappears by day 7. Although mice will undoubtedly feel discomfort at the inflammation in their knee, it is not sufficient to stop them moving, eating, or grooming. While the mice have arthritis we will measure the degree of swelling of both knees daily using callipers. On day 7 mice will be sacrificed so that we can investigate their immune system cells. Alternatively, we may sacrifice the mice at day 3 to investigate the immune cells at the height of disease.
To test whether different interventions can affect the severity of disease, or the function of cells of the immune system, we will usually begin treating mice before we give the first injection that causes disease. Treatment can be simply adding substances to their drinking water, however more commonly it will be given by oral gavage, or by intraperitoneal or intravenous injection. In oral gavage, a stainless-steel bulb tipped gavage needle, or a flexible cannula or tube, is attached to a syringe and used to deliver the compound into the stomach. For an intraperitoneal injection, a fine needle is used to inject the substance into the peritoneal space. Both these procedures are carried out while the mice are awake and being gently scruffed in one hand. Both procedures cause minimal distress when performed competently and properly, and may be performed daily for the course of the disease. For an intravenous injection the mice will be gently warmed up in a heating box to dilate the vein, which should cause minimal discomfort, and substances or cells injected into a vein in the tail. Generally, we will only give one intravenous injection.

What are the expected impacts and/or adverse effects for the animals during your project?

The major impact from the procedures in our licence will be the joint pain associated with arthritis-like disease. We have three models of inducible arthritis with slightly different durations of disease. Our most commonly used model involves joint swelling that peaks after 3 days and resolves after 7 days. Our second most commonly used model involves joint swelling that peaks after 6 days and resolves by 1518 days. Our third, and least used, inducible arthritis model can have joint swelling that lasts for a month, however we would typically cull these mice before that stage, as soon as we see differences between groups.
We will also use mice that display spontaneous arthritis which more closely models human disease. These mice can show joint swelling from 4 weeks of age that never fully recovers. We will generally cull these mice shortly after the peak of disease once differences between groups have been established (7-8 weeks of age).
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect the majority of animals that undergo arthritis in this licence to experience a moderate level of severity. The majority (~80%) of mice will be used for inducible arthritis protocols and these mice will experience a moderate level of severity or lower.
Mice that develop spontaneous, chronic, arthritis (~20% of mice used) will experience a moderate to severe level of severity due to the size and duration of joint swelling.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 12 April 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Part of the work in our group is human-based *in vitro* studies using peripheral mononuclear cells and serum from rheumatoid arthritis and systemic lupus erythematosus patients, which always informs our murine work. Unfortunately, at present we are unable to model systemic disease *in vitro*, as autoimmunity affects multiple organs via complex crosstalk between cell subsets, as well as cross talk between different physiological systems such as the mucosa of the gut and the joint. Answers that are more representative of human disease are obtained when we study directly the immunobiology of intestines, kidneys, liver, spleen, blood, lymph nodes and joints in diseased mice. This allows us to gain an overview of the potential of therapeutics. Presently we cannot do this either *in vitro* or *in silico*.

Which non-animal alternatives did you consider for use in this project?

Whenever possible we will use human patient samples and healthy controls to inform our animal work. Unfortunately, the only samples we can reliably obtain from humans are from the blood (peripheral mononuclear cells and serum) and therefore we cannot fully investigate the crosstalk of cells and the interaction between organs throughout the body.
To study the role of the gut in arthritis we have considered using cell lines such as the widely studied Caco-2 cell line, colonic cells that are derived from a colon cancer patient. However, we have found that the small intestine is more affected than the colon during arthritis, and thus the relevance of findings using Caco-2 cells to arthritis is unclear and would need to be confirmed using mice. However, if our future experiments determine that colonic cells are also of interest in arthritis we will, whenever possible, use this as an *in vitro* alternative to mice.

Why were they not suitable?

While human peripheral blood immune cells can be used to investigate very basic interactions between cells, or basic responses of cells to different substances, *in vitro* systems do not reflect the tissue specific behaviour of cells during autoimmunity. Cells recovered from the small intestine, colon, lymph nodes, or spleens contain different populations of white blood cell from those found in blood, and they can behave in quite different ways. In addition, although steps have been taken to try and model the anatomical structures where cells would interact *in vivo*, this work is still at a relatively early stage and the dynamics and duration of how cells may interact with each other *in vivo* in a lymph node, or in the gut, are quite different from how they will interact in liquid growth media in a test tube or well of a plastic plate. Thus, we still need to examine cellular interactions in a system that is biologically relevant to humans.
In terms of addressing basic interactions between white blood cells and the epithelial cells that make up the lining of the gut by using gut cells lines, the major problem is the origin of the cell lines. The majority of intestinal cell lines derive from colonic cancers and do no accurately reflect how the small intestine, the part of the gut that is most immunologically active, would respond. In addition, similarly to work with human white blood cells, growth of colonic cell lines in liquid on plastic does not reflect the anatomical structures these cells would comprise *in vivo*. Moreover, as joint swelling in human arthritis is the result of multiple immune phenomena, there is no specific readout (changes to a particular cell, or levels of a particular molecule) that can act as a surrogate of disease. Thus, to test the effects of a particular intervention on the development of arthritis we need to look at what happens to symptoms of disease in a complex biologically relevant system such as a mouse.

A retrospective assessment of replacement will be due by 12 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The large numbers of mice included in this project reflect the work of the 7 researchers employed in our research group. Careful consideration is given to minimise the number of mice needed to give statistically significant results. These numbers also reflect the fact that we are maintaining ~15 different strains of mice and as such have multiple breeding colonies.
Previous work in our group has provided pilot studies for most of our protocols and we have used them for power calculations. For instance, in the AIA model the power calculations showed that an experiment with at least 3 mice per group can reach statistical significance. We will perform similar calculations for all models in order to reduce the number of mice used overall for each protocol.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The size of the group has been decided after consultation with our departmental statistician and we will continue consultation for optimisation of the experiments throughout the life of the licence.
We will continue to consult with the NC3Rs' Experimental Design Assistant, as well as with the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use pilot studies to provide data for power calculations so that we can use the minimum number of animals necessary per experiment. In particular, when investigating novel treatments, we will use small pilot studies to determine the potential efficacy of the treatment so that no mice get used in the future if the treatment proves unsuccessful.
Where possible, we will combine experiments so that one control group can be used for multiple experiments and to allow for sharing of tissues from the same mouse between researchers.
We will also use inducible models of autoimmunity such as AIA or STA where possible, as these models have 90-100% incidence, minimising the number of animals needed per experiment.
A retrospective assessment of reduction will be due by 12 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques

during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The highly complex nature of human autoimmune diseases requires *in vivo* experimental models, and in order to better understand their heterogeneity we have included multiple murine models.
Arthritis models:

1. Antigen-induced arthritis (AIA) – moderate severity.

AIA animals are injected with methylated bovine serum albumin (mBSA) in complete Freund's adjuvant (CFA) subcutaneously followed one week later by an intra-articular injection of mBSA. This results in a self-remitting mono-articular knee swelling which resolves within 7 days. This will be our main model used for arthritis as it is probably the least severe model of this disease. In addition, AIA only affects one joint and the disease course is very short, both aspects limiting the suffering of the animal. AIA is an acute model of disease rather than a chronic model and therefore we require further arthritis models to investigate the chronicity of arthritis, such as the CIA inducible model or the K/BxA^{g7} mice which develop spontaneous arthritis.

2. Collagen-induced arthritis (CIA) – moderate severity.

CIA animals are immunised with type II collagen in CFA and rarely boosted with type II collagen in incomplete Freund's Adjuvant two weeks later. In contrast to the acute, short-term AIA model that we will use most often, CIA persists for up to a month and affects multiple limbs and joints within the same limb. These features make CIA a closer match to human disease than AIA and will provide us with a more representative system to confirm the potential therapeutics that we can initially test in the AIA model.

We will generate initial data in AIA and use the CIA model for confirmation so as to limit the time that mice experience arthritis.

3. Serum transfer arthritis (STA) – moderate severity.

STA animals are injected with serum from arthritic K/BxA^{g7} mice. This arthritis model is a good combination of the two above protocols as it is relatively short term (resolution by 15-18 days after serum injection) yet it induces inflammation in both rear and front paws, which is similar to human rheumatoid arthritis. Another advantage of this model is that it does not require the use of immunostimulatory adjuvants, such as CFA, which can cause localised discomfort for mice. However, the disadvantage of this model, which gives us the need for the adjuvant-induced models above, is that STA only activates part of the immune system and as such does not fully represent the human disease, which involves most cells in the immune system.

4. K/BxA^{g7} mice – severe classification.

Unlike the inducible models of arthritis, K/BxA^{g7} mice develop a spontaneous arthritis involving a loss of both T and B cell tolerance and production of autoantibody. The K/BxA^{g7} model will complement the AIA, CIA and STA models in our study as its spontaneous nature obviates the need for bacterial adjuvant or any other transferred substance to induce disease.

In terms of duration, K/BxA^{g7} mice will not recover from arthritis and we will humanely kill the mice as soon as we see reproducible differences between treatment groups.
As well as using arthritis models we will perform experiments in lupus and multiple sclerosis models to confirm whether our results are arthritis specific or can be generalised to autoimmunity more widely.
Systemic lupus erythematosus models

1. MRL/*lpr* mice – moderate severity.

This is a well-established spontaneous model of lupus that develops around 8 weeks of age caused by a mutation in a gene that controls cell death (*fas)*. We anticipate the development of autoantibodies and clinical symptoms, notably alopecia, proteinuria and rash, similarly to the clinical features seen in humans. Similarly to the arthritis models above, unfortunately we cannot replicate the complexity of lupus *in vitro* without the interaction of cells and physiological systems. We have used this model extensively and have shown that it is sensitive to changes in the B cell population, the cells we are studying.

2. NZBWF1/J mice - moderate severity.

NZBWF1/J develop an autoimmune disease resembling human systemic lupus erythematosus. Autoimmunity is characterized by high levels of antinuclear antibodies, hemolytic anemia, proteinuria, and progressive immune complex glomerulonephritis. The incidence and severity of symptoms is more pronounced in females. NZBWF1/J mice have been used as a model for autoimmune disease since the early 1960s elucidating not only the complex immunobiological responses and mechanisms but also the genetic basis for the complex multifactorial disease.

Multiple sclerosis model - Experimental autoimmune encephalomyelitis (EAE) – moderate severity.

EAE has been used by a number of research groups to study the role of Bregs in controlling autoimmunity. This model appears to be sensitive to both Breg control and the modulation of gut bacteria. Thus, it will allow us to investigate mechanistic aspects of regulatory B cell function and the role of microbiota in disease induction. Extending our work to a model of a third autoimmune disease will determine whether our findings on B cells, microbiota, or the gut itself are disease specific or whether they may apply across the autoimmune spectrum. EAE can induce total bilateral paralysis in mice, however on average most mice will only experience at most bilateral hind limb paralysis and should experience no pain. Mice showing any signs of front limb paralysis will be humanely culled before the disease can progress. EAE in mouse is the gold standard animal model for human MS as, similarly to human RA, it is mediated by both B and T cells, and induces myelin-specific antibodies.

Why can't you use animals that are less sentient?

Mice and humans are genetically and physiologically very similar. In terms of the study of autoimmunity this means that mouse models of disease have similar physiological characteristics and clinical symptoms to their human counterparts. As such, using mice as a model system for human autoimmunity has been proven to be very useful in research.

Unfortunately, animals at a more immature stage of life, or less sentient animals, are not as genetically or immunologically similar to humans and cannot therefore replicate human clinical symptoms as reliably. Furthermore, our models are carried out over several weeks and thus we cannot use terminally anaesthetised mice.

The availability of well described spontaneous and inducible models of autoimmunity makes mice currently the most appropriate species in which to carry out this work. In addition, it is now straightforward and affordable to generate transgenic mice to allow the precise dissection of the contribution of individual molecules to the immune system and disease. Thus, mice provide an excellent experimentally tractable system to investigate autoimmune disease development, immune responses, and therapies.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All protocols proposed in this application are well-published models that have been tried and tested to minimise discomfort. Anaesthesia and analgesia will be administered to minimise discomfort where appropriate, and animals will be assessed daily for any signs of distress including piloerection, hunched appearance, lethargy and loss of weight. In all the proposed *in vivo* models, if animals display any signs of distress, advice will be obtained from the named veterinary surgeon and, if distress cannot be alleviated, the animals will be humanely killed. Furthermore, we have chosen specific humane endpoints in order to limit the severity of disease models. Where appropriate, we will carry out pilot experiments to determine earliest endpoints of experiments so as to minimise suffering while generating reproducible data.

For instance, if we want to investigate the presence and function of long lived memory B cells (cells that are generated 2-3 weeks into an immune response) in one of our inducible autoimmunity models we will run initial experiments to determine when memory B cells first arise in that model. We will then design experiments that run just long enough to investigate these cells.

Lastly, all animals will be kept in well maintained, individually ventilated cages with sufficient bedding and nesting

material with good quality food and water, which will also reduce experimental variability caused by environmental stresses. Animals will be monitored for weight loss and signs of toxicity, pain or distress following the mouse grimace scale. In the event of unexpected adverse effects, we will use the humane endpoints written under every protocol.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence, as well as any other useful material, such as the Experimental Design assistant, from the NC3R website to ensure experiments are conducted in the most refined way. Where applicable we will also follow the refinements of arthritis protocols as outlined in Hawkins et al. *Applying refinement to the use of mice and rats in rheumatoid arthritis research*. Inflammopharmacology. 2015; 23(4): 131–150. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4508365/), and have incorporated a number of their refinements into our experimental protocols.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continue to consult with the NC3Rs website for any advances, as well as with the animal welfare officer, named veterinary surgeon and technicians from the animal facility.

A retrospective assessment of refinement will be due by 12 April 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

219. Understanding the mechanisms of ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b) **Key words**

ageing, reproduction, cancer, diet

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Ageing is defined as the continuous deterioration of an organism with age manifesting itself in the reduced reproductive output and a diminishing chance of survival. Why we age is still one of the highly debated questions

in biology, and several competing hypotheses exist. One hypothesis postulates that the separation of two major cell lineages - one for growth and maintenance (soma) and one for reproduction (germ line) - is a key factor in organismal ageing. The germ line as the producer of gametes and hence the subsequent generations is virtually immortal. This fact demonstrates that cell lineages may in principle continue forever. However, the tissues in the soma show less resistance to the passing of time and show continuous deterioration in their function and quality with increasing age. In contrast, germ cells show remarkable resistance and keep errors in the genome (mutations) during cell division and deterioration to a minimum. One possibility is that maintaining a cell lineage like the germ line is actually costly and comes at the price of a reduced maintenance in the soma. While a direct energy trade-off between the two is unclear, the importance of the interaction of the germ line and the soma for organismal ageing is indisputable. Nevertheless, very little is known about how the germ line interacts and communicates with the soma. While it is somehow intuitive that damage and stress negatively affecting the soma may also harm the cells in the germ line, it is less clear, how much the condition of the germ line affects the rest of the organism.
The ageing processes including the development of cancer in the soma are thought to be controlled by the germ line but the mechanisms involved are currently poorly understood. In recent years some dietary interventions including intermittent fasting and dietary supplements such as rapamycin have been suggested to be beneficial to slow down somatic ageing and potentially for slowing down the development of age related cancers. In this project we therefore will investigate the importance of germline-soma interaction for organismal ageing as well as the relative important of dietary interventions such as intermittent fasting and rapamycin in the zebrafish.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Understanding the mechanisms of ageing helps us to tackle the societal problems of increasingly ageing populations and understand the causes of age-related diseases such as cancer.

What outputs do you think you will see at the end of this project?

This project will produce some highly novel data on the mechanisms of ageing. Signs of organismal ageing can be observed in the accumulation of mutations (i.e. changes to the genomic code) in different tissues, in alterations of the epigenome (i.e. non-genetoc components of the genome e.g. the methylome) as well as in changes in gene expression in various tissues over a lifetime. In addition, reproductive success is a good indicator of general organismal fitness and declines rapidly with age. We will collect data on mutation rate, methylation rate, gene expression and reproductive output and cancer development in wild-type and cancer mutant fish, with and without germ line as well as with and without dietary interventions such as supplementation or intermittent fasting. All these results will provide information that is directly translatable to similar interventions in humans and other vertebrates and will be of wide interest.

Who or what will benefit from these outputs, and how?

Understanding the mechanisms of ageing and age related diseases such as cancer and how to intervene has far-reaching consequences of our rapidly ageing society. Testing simple interventions such as existing dietary supplements and dietary restriction in treatment of ageing and cancer promises to be of widest possible interest. As one grant reviewer has put it recently: “Who would not want an anti-ageing pill".

How will you look to maximise the outputs of this work?

We will disseminate our results through publications in internationally renowned journals, at national and international workshops, conferences and at events involving the general public and through the press. Our lab has a history of publishing also null-results and we apply an open and communicative policy to share our experiences with other labs, the wider scientific community as well as the general public. We are collaborating with a number of research groups across the globe and will continue to do so.

Species and numbers of animals expected to be used

- Zebra fish: 6600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use the zebrafish as much of the ageing research to date has been performed in invertebrate organisms such as C. elegans and Drosophila flies. These invertebrate organisms differ in key biological aspects from vertebrates which affect the ageing processes in the two in very different ways. In order for studies to be directly translatable to other vertebrates including humans, the use of a vertebrate model is necessary. No other, less sentient vertebrate is better suited than the zebrafish. We will use all life stages, as we study ageing throughout life.

Typically, what will be done to an animal used in your project?

Genetically Altered (GA) fish of well-established mutant strains (cancer mutants and germline-ablation mutants) will be bred and monitored throughout life.
One strain of the project simply aims to collect fish at different ages for follow-up studies in their tissues for signs of ageing and cancer development by histological analyses and sequencing.
The second strain aims to understand how mild dietary interventions such as dietary restriction through intermittent fasting and the supplementary feeding of rapamycin may stall or halt the signs of ageing and related cancer.
For both purposes, fish will mainly be maintained under the different treatment until a maximum age of 24 months.
Fish may be anaesthetised up to five times for the collection of finclips or for skin swabbing. These procedures will occur at a maximum frequency of every six months.

What are the expected impacts and/or adverse effects for the animals during your project?

Fish in this project belong to GA mutant strains which result in germline ablation or the development of cancer phenotypes. All these strains are already established and the phenotypes and onset of phenotypes well established. All fish used in this project will not experience any severity level beyond mild. The GA mutant strains developing cancer phenotypes will be carefully monitored. The onset of cancer in these strains is well known and we will end our experiments at an early stage to collect tissues for sequencing and histological analyses.
The dietary interventions such as dietary restriction and supplementary feeding of rapamycin are expected to improve the overall health of any fish, and more so in mutant strains of the types mentioned above. So if anything, we expect beneficial effects from these treatments.
All other procedures such as anaesthesia, fin clippling, skin swabs and gamete collection are standard procedures that have been practised a lot in our lab.
Anaesthesia is not expected to have any harmful effects. Fish that do not return to normal swimming behaviour within 30 minutes after removal of the anaesthetic will be killed by a schedule 1 method.
Genotyping is generally limited to a skin swap, and where necessary to a finclip. Following fin clipping for genotyping analgesia will be provided to reduce potential pain. Any fish exhibiting any abnormal behaviour following skin swabs or finclips will be killed by a schedule 1 method.
Infections can result from fin clipping (<1%) or from damage to scales or loss of mucous surface from swabbing. The procedure will be carried out using sterile equipment. Fish that develop signs associated with infection will be killed by a schedule 1 method.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect mild severities at all stages and for all protocols as any other categories will be euthanised immediately.
What will happen to animals at the end of this project?

- Kept alive
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The complexity and fundamental nature of studying the processes involved inn ageing and the development of ageing related diseases such as cancer currently still requires the use of animals. The reason is that the mechanisms of ageing are not yet fully understood and this project will considerably contribute to our understanding of these mechanisms. Using the zebrafish as the lowest sentient vertebrate model species allows us gathering insights into ageing in vertebrates that are directly translatable to other vertebrates including humans.

Which non-animal alternatives did you consider for use in this project?

Invertebrate model species such as C. elegans and Drosophila fruit-flies are widely used in the study of ageing.

Why were they not suitable?

However, the fact that their somatic tissue no longer grows during adulthood changes the interaction between germline and soma for the process of ageing decisively. It is therefore necessary to study these mechanisms ins a vertebrate, and the zebrafish belongs to a low sentient category.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These numbers are based on our previous experience with similar protocols. We worked out how many fish we will need to answer our scientific questions and then calculated how many fish will need to be used to generate those experimental fish.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have been breeding zebrafish for quite some time and know that a minimum stock population is needed to maintain a healthy population in the lab. Many of the GA lines that we will use are stocked as frozen gametes at zebrafish breeding centres. But in order to use these, we will have to get in the embryos rear them to adulthood and then use the next generation of fish for our experiment.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We aim to maintain our fish under optimal conditions in the lab and are continuously informing ourselves about improved feeding and maintenance protocols as well as protocols to collect tissue samples for genotyping etc.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

All the treatments in the protocols involve mild severity levels. In fact, some of the treatments are expected to have beneficial effects (e.g. dietary intervention). We will end the experiments at the earliest possible time points when cancer pehnotypes, which are the most harmful expected phenotypes start developing. This means we keep even these potentially harmful cancer phenotypes to a minimum.
All other procedures are standard procedures and are only carried out by highly trained staff.

Why can’t you use animals that are less sentient?

Invertebrate model species such as C. elegans or Drosophila fruitlfies lack some of the key biological aspects that are necessary to study the role of the interaction between germ line and some in vertebrates, namely a growing proliferating soma. The zebrafish is in the least sentient vertebrate category.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will carefully monitor all animals generated, bred and maintained under this license. Any animals showing signs of suffering or suboptimal behaviour will be immediately removed and killed under schedule 1 procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We closely follow any guidance by leading institutions in zebrafish breeding and husbandry.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We keep ourselves updated by visiting relevant conferences and taking useful online courses.

Home Office

NON-TECHNICAL SUMMARY

220. Understanding the replication of and immune responses to coronaviruses in pigs

Project duration

1 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

coronavirus, respiratory disease, virus replication, immune responses

Animal types	Life stages
Pigs	juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to establish a model of porcine respiratory coronavirus (PRCV) infection in pigs to investigate protective immune responses to infection and vaccination. This model will help understand protective

immune responses to coronaviruses, with relevance to SARS-CoV-2 infection of humans.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The coronavirus responsible for the COVID-19 pandemic, SARS-CoV-2, is a new virus about which very little is known. Animal models are being developed which show susceptibility to infection, however the virus is not known to naturally occur in any of them. Investigating the immune responses to both infection and vaccination of a naturally occurring respiratory coronavirus in pigs, would allow detailed host responses to be defined and help understand the biology of respiratory coronaviruses.

What outputs do you think you will see at the end of this project?

The output from this project will be development of a porcine respiratory coronavirus model which mirrors the pathology seen in COVID-19 / SARS-CoV-2 infection of humans. This model would then be available to study various aspects of vaccinology, immunogenicity and therapeutic interventions as a tool to assist COVID-19 research.

Who or what will benefit from these outputs, and how?

Novel therapeutic and prophylactic anti-viral solutions are urgently required to control the spread of emerging coronaviruses including SARS-CoV-2. An understanding of coronavirus replication and the host responses to coronavirus infection will inform future strategies to protect the human and livestock populations from potential new incursions of novel coronaviruses.

This project aims to develop porcine respiratory coronavirus as an animal model to understand more about how coronaviruses cause disease and the immune responses that are raised against coronaviruses and vaccines in the natural host.

Our studies will benefit researchers working in the fields of molecular virology, virus evolution and particularly, coronavirus research. However, it will also be important to medical researchers investigating antiviral therapies.

How will you look to maximise the outputs of this work?

The data will be shared in open-access data repositories a soon as practicable to allow other researchers to benefit from these results. Knowledge generated by this project will be widely disseminated to the research community as soon as practicable through open-access peer-reviewed journals and presentations at national and international virology conferences, collaborative discussions and interactions with members of the scientific community. Conference attendance will allow the dissemination of results and facilitate collaborative discussions, enhancing potential outputs from the proposed project.

Species and numbers of animals expected to be used

- Pigs: 18

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Pigs are being used as they are the natural host for porcine respiratory coronavirus (PRCV), and this infection has previously been shown to produce very similar lung disease as SARS (and by extension COVID-19). The ages of pigs being used are in line with previously published literature.
Typically, what will be done to an animal used in your project?

The duration of the pilot experiment is likely to be 14 day maximum, and will involve sedating the pig then taking swabs and a blood sample. Whilst sedated, the pig will have virus administered into the nose directly, and into the trachea via the mouth, or virus will be administered by aerosol using a closefitting face mask. The sedation will wear off and the animal will be kept for up to 14 days, and monitored closely at least 2 times a day. Swabs and blood samples will be taken from the animal between being inoculated and being culled at the end of the study. At the end of the study, the pigs will be killed by administering an overdose of anaesthetic.
What are the expected impacts and/or adverse effects for the animals during your project?

Animals will experience mild and transient pain associated with a blood sample being taken, which includes restraint and insertion of a needle through the skin. The same level of discomfort will be experienced by those pigs which are sedated. The effects of PRCV in pigs are mainly either subclinical or mild. The mild signs will be intermittent coughing or a mild elevation in body temperature. Relatively rarely, animals infected with PRCV will have difficulty breathing, become lethargic or anorexic. In this project, any animals which show these more severe signs will be killed if the duration exceeded the endpoints in this licence.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

According to the cumulative literature, 95% of animals are expected to show either no or mild clinical signs. Less than 5% are expected to show moderate clinical signs but one of the objectives in the pilot is to develop the PRCV model and evaluate the clinical signs in pigs.
What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are required to study the immune responses to virus infections.
Which non-animal alternatives did you consider for use in this project?

Cell culture and molecular biology techniques will be used to characterise PRCV in the laboratory before infecting pigs. We will use laboratory techniques to understand the responses of the pigs to infection with PRCV using blood samples and tissue samples taken post mortem.
Why were they not suitable?

It is not possible to analyse immune responses to virus infection without using a host animal because the immune system is very complex.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used

throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of pigs in this pilot study is based on the available literature.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

As this is a pilot study, the design of the experiment is based on the published literature on this subject.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

This pilot study will be used to optimise the number of pigs required for future experiments.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will take blood samples from pigs, sedate them to avoid causing distress and infect them with a porcine coronavirus. According to the available literature, most of the pigs will experience mild clinical signs. They will be closely monitored by experienced staff throughout the study. Blood samples will be taken at regular intervals and the pigs will be killed at the end of the study to evaluate the pathology caused by the virus and assess the immune responses in the pigs. The blood samples will not cause lasting distress or harm to the pigs.
Why can’t you use animals that are less sentient?

Pigs are a good model for human respiratory disease due to their size and the shape of their lungs. They are also a natural host of a respiratory coronavirus.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

This is a pilot study and the pigs will be monitored closely by experienced staff throughout the experiment. The results of this study will be used to inform future experimental design.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow guidelines from the NC3Rs.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will consult the AWERB for advice about the 3Rs and participate in the annual 3Rs workshops organised onsite.

Home Office

NON-TECHNICAL SUMMARY

221. Understanding the role of the body clock in behaviour and physiology

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, pregnant, juvenile, neonate, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this work is to define how circadian timing systems (our bodies' internal clocks) regulate rhythmic physiology, energy metabolism, and our overall cardiovascular and metabolic health.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The circadian timing system regulates daily rhythms in virtually all aspects of our biology, from sleep/wake timing and eating habits, through to how individual cells of the body produce and use energy. Uncovering how this endogenous clock system works is fundamental to understanding not only our basic biology, but also how our health is impacted by aging and the pressures of modern life (which can undermine the normal function of our body clock). It is becoming increasingly clear that disruption of our circadian clock is detrimental to health and well-being, and has been linked to numerous disease states ranging from cancer to metabolic diseases, including type 2 diabetes and cardiovascular disease. Our work examines this link between the clock and disease states at both a cellular and whole organism level. These studies continue to reveal important events which drive pathology and identify novel approaches to improving human health and well-being.

What outputs do you think you will see at the end of this project?

Major Benefits. The primary outcome and benefit of this project is the advancement in understanding of how the biological timing mechanisms regulate physiology and behaviour. A specific context of our work is in the development of metabolic diseases, such as type 2 diabetes and cardiovascular disease, and how disruption of our bodies' clock system (e.g. through aspects of modern life such as shift work) contributes to these conditions. Thus, a primary output from this work is knowledge creation, which will be delivered through publication in academic journals, conference seminars, and communication with regulatory and advisory committees, the clinical community and wider public.

The potential for this research to benefit human and animal health is high, given the scale of the unmet need in clinical medicine for new ways to deal with metabolic diseases and pathologies associated with circadian dysfunction. Our work will identify key cell types and molecular pathways that initiate and propagate such disease processes. These basic science advances are essential in the long-term for medical breakthrough and therapeutic advancements. Development of therapeutic interventions (both pharmacological and non-pharmacological) which target the circadian rhythm to improve well-being is achievable. For example, outputs from our work have, and will continue to inform on the use of timed feeding, specific light interventions and improvement of behavioural routine to achieve health benefits. Benefits will include the identification of molecular targets at which new pharmaceutical products could be aimed.
The research also benefits the health and welfare of animal in the laboratory, farm and at home by providing better understanding of how environmental conditions impact on the circadian clock, and in turn downstream clock-related physiology.
Additional outputs are likely to include the development of new research methods and protocols. This may be in the form of refining and/or enhancing current methods, or through the development of new tools and approaches. Our data will be made available to other researchers in the scientific community at the earliest appropriate time, and therefore enable further/secondary use of the outputs for scientific discovery.

Who or what will benefit from these outputs, and how?

Our internal body clock is fundamental to our biology, and how we live our lives (e.g. when we eat, sleep, work, stare at computer or phone screens…) can profoundly affect our health. Our work will define new aspects of cardiovascular and metabolic physiology, reveal mechanisms of circadian clock control over physiology, and determine how clock function (and dysfunction) contributes to metabolic disease. The outcomes will inform the scientific community, health care sector, occupational health regulators, and government/industry advisory boards,

and the public in general, in terms of both understanding the potential positive/negative impacts of daily routine on health, but also how light and/or food may be used to alleviate such consequences.

How will you look to maximise the outputs of this work?

We will engage with the wider scientific and clinical community through presentations at major national and international scientific meetings, and via high profile publications. To ensure the outcomes of our basic science research achieve maximum benefit, we will focus on communication and engagement with key stakeholders (healthcare sector, regulators, policy makers), the public, and relevant patient groups. We have a strong commitment to Public Understanding of Science activities, in maintaining public interest and trust in UK research, and informing the public on the latest research findings. **Species and numbers of animals expected to be used**

- Mice: 13500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use adult mice for the vast majority of studies undertaken within this project. This includes normal control and genetically modified mice. Importantly, the systems that we are studying (the body clock, regulation of energy metabolism, and development of obesity) are well reproduced between mice and humans, and mice are therefore appropriate for this work.

Typically, what will be done to an animal used in your project?

Common procedures (~65% of the mice that we study will undergo one or more of these procedures). **i**) Animals may be monitored for natural behaviours under normal or altered environmental conditions (e.g. alterations in light/dark cycle, modulation of ambient temperature across a natural range, altered patterns of food availability). **ii**) Animal may undergo physiological monitoring and/or imaging using non-invasive specialist equipment. **iii**) Animals may also receive injections or have small blood samples collected. **iv**) Animals may have the amount, composition (e.g. increased fat content) or timing of their food changed.

Rare procedures (~12% of the mice that we study will undergo one or more of these procedures). **i**) Surgery in order to insert a device for physiological monitoring, slow release drug delivery, or to induce a change in gene expression (minor surgery with rapid recover of typically 1-3 days). **ii**) experience changes in ambient temperature (within a naturally occurring range) to assess adaptive metabolism.

In addition to these procedures, mice will be used in this project for breeding, and for provision of cells and tissues for laboratory based studies.

What are the expected impacts and/or adverse effects for the animals during your project?

Much of this work examines normal behaviours and physiological processes in the animals. Therefore, most animals experience only transient and mild adverse impact (e.g. temporary stress, discomfort, and/or weight loss) caused by alterations in their housing environment (e.g. changes in lighting cycle, ambient temperature, timing of food availability) and/or cage type. Removing food access for 1-2 days may lead to more pronounced weight loss, but this is rapidly recovered upon re-feeding.

Some animals used here will also experience transient discomfort and mild pain due to surgical implantation of monitoring devices and/or injections.

Alterations in diet composition may lead to weight gain and obesity. Over time (~4 months in mice), obesity may lead to insulin resistance and low level inflammation, akin to that observed in individuals with type 2 diabetes. This may cause subtle changes in behaviour (e.g. reduced activity), but is not associated with any suffering or distress.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The studies to be undertaken in this project will result in a cumulative impact to the animals that are of a sub-threshold to mild (~60% of animals used) or moderate (~40% of animals used) severity rating.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The diseases we are studying involve the complex interaction between multiple organ systems, and often progress over long periods of time.

Which non-animal alternatives did you consider for use in this project?

We routinely use in vitro (e.g. using cells) and information from human data resources (e.g. UK BioBank). In vitro cell/tissue assays can offer powerful models to test how genetic or pharmacological alteration of cell function may impact on clock or metabolic activity and vice versa, and can inform on the best design of subsequent in vivo animal experiments. The complex neural pathways controlling sleep and metabolism however make it unlikely that such approaches can replace the need to use animals. Human studies are important for informing our work, but cannot replace the need to do mechanistic studies in animals.

Why were they not suitable?

We always explore alternatives to using animals in research, as well as developing methods which allow us to use fewer animals to achieve the same scientific objective. However, since circadian biology and metabolic diseases involve multiple tissue systems acting across the body, it is impossible to wholly mimic this biology in a culture dish or by computer.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have extensive experience with these approaches and in running projects of similar scope. Thus, estimates of animal use is based on i) previous work and experience with the methodologies used, the physiological parameters to be studied, and the specific mouse models to be used; ii) the scope and objectives of the current project; and iii) careful consideration of experimental design.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The projected number of animals used over the 5-year duration of the licence is an estimate. At each stage of the project and within each study conducted, we will ensure that animal use is minimized. We employ careful planning

of experimental design including the use of supporting purpose written software (including the National Centre for the Replacement, Refinement and Reduction of animals in research, Experimental Design Assistant). We have consulted with statisticians in preparation of this licence, and will continue to do so throughout the project.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In addition to good experimental design, we work to optimise animal use wherever possible. This starts with efficient breeding of transgenic mouse lines. For example, where possible we use 'wave breeding' techniques to minimise age variation and breeding numbers, while achieving adequately powered group sizes. Where new approaches are being employed, we optimise experimental conditions through pilot studies. Wherever possible, we share tissues, data, and results with other research groups to maximise the outcome and use of each animal study.
Moreover, through continuous development of our approaches we seek to reduce the use of animals. For example, our development of novel mouse lines which allow longitudinal measures of gene or protein expression (via bioluminescence) to be collected from individual animals rather than requiring many animals to be studies at numerous discrete time points.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project focuses on mammalian physiology with all our studies being conducted in mice. Our scientific objectives require us to examine whole animal physiology in adult animals over both short and long-term time frames. The methods we use are, for the most part, non-invasive or involve only temporary discomfort due to surgical implantation of a monitoring device (e.g. to monitor heart rate). Animals may also be provided with alternative diets (e.g. to drive obesity), but these conditions do not themselves lead to suffering and/or distress. To minimise any impact to the animals, we always end the studies as soon as the study objectives have been met. Our researchers are well trained and experienced in animal behaviour and physiology; this reduces stress to the animals under study, and ensures that animals are well monitored and cared for.
Why can’t you use animals that are less sentient?

We cannot adequately replace these studies with other species (e.g. insects or fish) or with very young (neonate) mice and achieve these objectives. As most of our studies involve long-term assessment, they cannot be conducted in terminally anaethetised animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

As we study natural physiological responses and behaviours in the animals, minimising stress, discomfort and other general impacts is critical to our work. We take many steps to minimise adverse impact, including acclimatisation of the animals to our researchers and any test conditions (e.g. new cages) during study. Examples of relatively simple, yet highly effective refinements include using appropriate handling techniques (e.g. tube handling for cage extraction) and use of appropriate home cage enrichment. Where animals do undergo invasive treatments (e.g. surgery to implant monitoring devise), we ensure full recovery of the animals under close monitoring and post-operative care to minimise any suffering (e.g. pain management with analgesics, provision of extra fluids). Where appropriate we will assess whether acute studies can be undertaken under general anaesthetic rather than in conscious mice (e.g. acute ECG assessments). Examples of our past and ongoing refinement of approach include increase efficiency in breeding through the use of conditional and inducible

transgenics (which allow for the activation or deletion of genes in specific cells and tissues at specific times), development of genetic reporters to reduce animal use, transition to advanced technical approaches which provide higher quality of information with less impact to the animals, and development of sophisticated analyses to maximise the data obtained from in vivo studies.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will ensure best-practice through continual assessment of experimental design and study outcomes, relative to the cumulative impact to the animals. We will stay informed about regulatory standards (e.g. LASA and NC3Rs publications) and developments within the scientific community (e.g. publications on standardisation and best practice for metabolic phenotyping of mice).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay informed about advances in 3R approaches, and will continue to implement these into our work where appropriate. This includes, but is not limited to, staying up to date with NC3Rs recommendations, developments in the literature and scientific community, as well as through our own developing work and discussions with our NVS and NATCO.

Home Office

NON-TECHNICAL SUMMARY

222. Using bioelectronics to study the nervous system and to restore neurological functions.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Brain, Spine, Nerves, Technology, Disability

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

In the world over 1 billion people are affected by a neurological disability (for example the inability to move a limb or talk, blindness or deafness, etc.), due to either brain/spinal cord injuries, stroke, cancer, etc. Most of these disabilities lack specific and effective treatments and medical efforts are generally placed in supportive care rather than cure.
'Bioelectronics' (small electric devices implanted in the body) offers a valid option to treat many of these diseases by interacting with the body, sensing what is wrong and substituting and restoring lost functions. An example is curing paralysis (inability to move a leg) following a spinal cord injury. Usually the signal to move a leg starts from

our brain, it goes through the spinal cord in our back bone to then reach our leg muscles. However, when our spinal cord is injured after injury to our backbone, it cannot recover, and therefore the signals do not pass through anymore. With 'bioelectronics', we can transfer information directly from the brain to the limbs, bypassing the spinal cord injury, and therefore restoring leg function.
To achieve this important result, we have to study four aspects of the process:
1 – How these devices can control and substitute body functions effectively.

2 – How the devices can sense what is wrong with our body.

3 – How to connect these devices and 'talk' between them to bypass sites of injuries.

4 – How to make these devices stay in the body for a long time without breaking or losing their activity.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This new type of technology will offer tangible treatment for multiple diseases of the brain, spine and nerves, which are untreatable at present. This includes paralysis, Parkinson's Disease, epilepsy and brain/spinal cord injury. Additionally, these innovative devices will allow us to find out more about diseases of which we do not know much about, so increasing the chance to find new treatments.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

8000 Rats and 3050 Mice Over five
years period

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

A third of the animals in this project license will have an intervention during terminal anaesthesia (animal humanely killed before waking up) and therefore not having any discomfort. These types of interventions are used wherever possible and they are important to collect information used to minimise and optimise intervention in animals which are woken up after the intervention.
The rest of the animals will undergo surgery with recovery (woken up after receiving an intervention), with a moderate discomfort in the immediate post-operative period, during which pain killers will be given. Post-surgery, they may show reduced or altered movements, where they have received a lesion to mimic an aspect of the human disease. These animals may later receive a treatment to repair the lesion, such as neural device implantation, which will assess the effectiveness of bioelectronics to ameliorate the effect of the disease model. Animals are expected to have mild to no lasting adverse effects after they have recovered from the surgery itself. Some animals will undergo mild food restriction to motivate their performance in behavioural tasks which are reward-driven, and they will be carefully monitored to ensure that no sign of dehydration occurs, and any weight loss is kept to less than 10% of their adult body weight. We expect no adverse effects due to the behavioural testing.

At the end of all experiments animals will be killed, and in most cases, tissues taken for more testing in the laboratory.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

This research is only possible because we need to study whole nervous system diseases as we do in patients. Human studies in patient groups are used as much as possible, with functional imaging and post-mortem studies but new therapies to arrest the disease can only be studied using animal models because of the experimental nature of the devices being tried.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We carefully design our experiments such that we maximise the behavioural and tissue data collected from each animal. We use the minimum number of animals required to yield statistically and biologically meaningful data, and we will work towards designing more refined behavioural tests such that the number of animals needed to generate this data can be reduced.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Rats are the best experimental animal for neural interface device development because the brain/spinal cord/nerves are reasonably large, there is sufficient space in the body or under the skin for connectors and interfaces, and the patterns of nerve impulses in response to movement are similar to those in humans.
Mice are used when it is important to use genetically modified animals, particularly in experiments in which we are working out which mechanisms in the immune system are responsible for scarring in response to prostheses.
Our devices will first be developed in vivo in non-recovery testing models and subcutaneous implantation models. These models entail a lower degree of harm for the animals, and ensure that only devices which are working as intended are progressed on to the neural implantation models.
All experiments are done on one side of the animal only, to minimise disability.
Furthermore, rats and mice have a biology of nervous system damage, repair and neuronal growth similar to humans. Rats are also capable of complex behaviour and skilled paw use, making it possible to achieve good behavioural outcomes. Mice are used for some experiments because they can have their DNA manipulated. Their behaviour is almost as good as that of rats.

We minimise suffering by developing and/or using behavioural outcome tests of high resolution that pick-up deficits in fine movement control. Therefore, it is not necessary to make large and disabling injuries. We use well established lesion models that have been extensively used in our lab for a number of years, thus we have achieved a high rate of reproducibility.

Home Office

NON-TECHNICAL SUMMARY

223. Using mouse models of dementia to understand disease mechanisms

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To characterise mouse models of dementia causing diseases to understand underlying cellular and molecular mechanisms. Proof of principle drug, genetic intervention and environmental intervention studies to test the potential of possible therapies to ameliorate dementia-associated phenotypes.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Today about 50 million people world-wide have dementia globally. There is no cure for the diseases that cause dementia and current treatments for disease are largely ineffective. Dementia-causing diseases are increasing in occurrence globally because of rising life-expectancy and they cause a huge societal and economic burden world-wide. Despite identification of some of the genetic changes that cause dementia-resulting disease over the last 3-decades; how these genetic changes result in the molecular and cellular changes that underlie dementia is still not well understood. Thus, further medical research is required to understand how changes in the brain and body cause a decline in the ability of people to think and remember, so new therapies can be developed.

What outputs do you think you will see at the end of this project?

The primary objectives of this project are to provide new and well characterised models of dementia-causing

- diseases, further understanding of the causes of dementia-related phenotypes, to undertake proof-of-
- principle studies of potential therapies for dementia-related phenotypes.
- New models generated on other projects will be characterised in this project to full-fill current gaps in preclinical modelling of dementia-causing diseases, including identification of novel therapeutic targets and the determining the mechanisms underlying biomakers used for early diagnostics.

We aim to define the order in which dementia-relevant phenotypes occur in a series of mouse models of dementia causing disease; including those not previously studied. Specifically, we will undertake in depth phenotyping of 5-10 preclinical mouse models of dementia causing diseases, to provide new understanding of common pathways to dementia, as well as disease specific biology.
Firstly, by the identification of specific genes, changes in genes or chromosomal regions that modulate dementia-related phenotypes. Secondly, by the identification of sub-types of cells in which this genetic variation acts. Thirdly, to determine how environmental and pharmacological interventions can modulate dementia-related phenotypes. These data will lead to the identification of potential targets for therapy and preclinical model biomarkers. These will then need further evaluation in human cellular models (including iPSC and organoids), human post-mortem tissues and were possible in clinical datasets. This work will be undertaken by the project licensee in collaboration with others and also independently by other researchers. This combined knowledge can then be used to develop new treatments to slow or prevent the development of dementia. The complexity of developing new treatments for brain diseases makes it likely that new therapies will not be developed within the 5-years of this project.
These new research findings will be published in the primary literature and also all testing protocols will be made freely available.

Who or what will benefit from these outputs, and how?

In the short-term this project will supply mice and tissues to other researchers, including from genetic crosses, animals that have undergone drug treatment or changes to the environment and new mouse models characterised during this project. This will refine and accelerate the research of these investigators.
In the short-term other researchers will also benefit from outputs in the primary literature; both directly produced from this project and as a result of collaborations with other researchers. We will also deposit primary data onto established data repositories were possible.

This research project will contribute to a refinement of mouse models and an improvement in the value of research outputs using these models within the 5-years of this project. In particular, the detailed characterisation of new mouse models will benefit other researchers. For examples, researchers studying behavioural changes will benefit from the our detailed studied of sensory, motor and metabolism changes which will allow them to refine their research by controlling for these factors in future studies if required. Researchers focusing on molecular, cellular and electrophysiological causes of dementia will be able to use the data from this project to help identify the model that is most appropriate for their research question.
We will also produce data determine the role of specific molecular and cellular causes of behavioural changes associated with dementia. These new pathways can be tested to determine if they can be modified by drug therapies to delay or prevent dementia-associated cognitive and behavioural changes. Thus this basic/translational research projects outputs will benefit dementia patients and their carers' and wider-society in the long-term.

How will you look to maximise the outputs of this work?

We will disseminate all new knowledge internally and externally by publication in the scientific literature. This will include the results of all phenotyping studies that passed internal quality control checks. We will actively collaborate with other leading dementia-diseases mouse model researchers and attend international conferences to further disseminate research findings internationally.
Species and numbers of animals expected to be used

- Mice: 54000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Breeding mouse models of dementia-causing diseases with other models will allow us to link molecular and cellular changes to specific dementia-relevant phenotypes. In particular, we can study the causes of dementia-related apathy/ decline in motivation/depression, executive dysfunction (loss of ability to coordinate and control behaviour) and loss of sub-types of memory in mice, which would not be possible in invertebrate models or *in vitro* systems.
Also, we can study how changing the animal's environment and or genes alters processes in body, that are thought to be important to dementia (such blood pressure or diabetes) and how this might affect dementia. Similarly, we can study how dementia-associated neuropathology affects physiology of the body (such as hearing and sight). Studies in mice also allow the interaction of different dementia causing disease that co-occur in patients (e.g. vascular dementia and AD) to be better understood, and whether these co-morbidities are linked by common mechanisms.
Dementia risk is increased by ageing and thus it necessary for us to undertake our research in mature adults that have a fully developed central nervous system.

Typically, what will be done to an animal used in your project?

Many of the mice on this licence will be used only for breeding. This is due to the complex nature of dementia causing diseases and our aim to understand the genes and cells that affect disease. This requires several steps of breeding to get to a group of mice with the necessary combination of genes that can be studied. We will also maintain breeding lines to ship to collaborators for use on other projects authorized to accept GA lines, with the aim of reducing overall animal use.
Approximately 14,000 mice will undergo a combination of phenotyping tests to characterise preclinical models and

for hypothesis-testing. Each experiment will use a combination of tests over the life-span of the animals, most of which are non-invasive, a small number involved anaesthesia used for immobilisation, for imaging, physiology recordings or the administration of substances to modulate gene expression.
Typically mice are then finally anaesthetised and a terminal bleed or perfusion carried out. All experiments are expected to end by 104 weeks of age, with the majority of mice being culled by 78 weeks of age.

What are the expected impacts and/or adverse effects for the animals during your project?

Genetic alterations in the mice used in this project may lead to the development of dementia-relevant phenotypes. In mice these changes may lead to the increased aggression/fighting, decreased food intake leading to weight loss and altered response to some procedures. For example, impaired wound healing after surgery because of impaired immune system may occur in some lines used in this project. Also these genetic alterations will lead to changes in memory and behaviour that may not be apparent in the home-cage but require additional testing to measure.
Mice undergoing cognitive testing using a food-reward for motivation will have reduced access to food to reduce their body-weight to ~90 % of their free-feeding weight. Studies have shown that in some knock-in mouse models of dementia causing disease, food restriction increases survival, consistent with data for C57BL/6 mice in general. Thus we do not anticipate adverse welfare outcomes associated with food restriction. To improve the validity of our results we will monitor long-term effects of food restriction by assessment of blood glucose before and after food restriction and body mass composition.
For some tests mice will need to undergo anaesthesia (e.g. for surgery for the implantation of devices for remote monitoring, hearing response assessment and for the injection of substances into the brain). All surgery and anaesthetic use in mice carries a risk of mortality and a risk of pain (short-term and long-term), which may differ between genetically altered lines. Injection of substances into the brain carries a risk of rare adverse effects such as inflammation, seizures and a risk of wound reopening. In pups injection of substances requires separation from the mother which increases the risk of maternal rejection of the whole litter.
Blood sampling requires the use of restraint to ensure safe sampling in a controlled manner. This poses the risk of induction of a stress response. The use of assays which minimise the required sample volume will be used and larger volumes of blood will not be sampled when the mice are on restricted food intake protocols.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

10,000 mice on the mild breeding protocol are not expected to suffer any adverse effects and the vast majority will not reach higher than a sub-threshold severity.
On the moderate breeding protocol, it is anticipated that any mice carrying the disease-causing phenotype could exhibit a moderate phenotype. Other genotypes will also be born from these crosses, so approximately 25% of the 30,000 mice may suffer a moderate severity.
14,000 mice on the behavioural phenotyping protocol (P3) are all expected to reach a moderate severity. This is partly due to the phenotype of the mice, in which the genetic alteration could lead to a moderate severity in around 50% of the mice (the other 50% being unaffected controls). However, all mice will reach a moderate severity because of a subset of the phenotyping tests causing moderate suffering, for example overnight fasting for blood sampling or food restriction for the purpose of motivation in food-rewarded tasks. Additionally, the combinatorial effect of repeating mild tests over the life span of the animal, in order to understand the progression of disease, will lead to an overall moderate experience. This combinatorial effect of repeating mild-phenotyping tests may also interact with genetic alterations used in this project to model dementia causing-diseases. Whilst these moderate affects will be short lasting they will increase the maximum severity of all animals on this protocol to moderate.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The primary objectives of this project are to

- link molecular and cellular changes of dementia-causing diseases to dementia-relevant phenotypes (including changes to cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole body physiology),

- understand how environmental changes and pharmacological interventions can modulate dementia-relevant phenotypes.

It is not possible to manipulate the molecular and cellular pathways in people who have dementia in order to determine how these processes impact on cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole body physiology. Thus an animal model must be used.
The wider research community is undertaking significant work analysing clinical and human postmortem datasets from people who had dementia-caused diseases, preclinical work in cell, organoid and invertebrate model systems of dementia-caused diseases.
This project will link with and draw on these experiments to minimise animal use and maximize translation value of the preclinical mouse work in this project. The work in this project cannot be addressed elsewhere using alternative approaches.

Which non-animal alternatives did you consider for use in this project?

Clinical longitudinal studies of people who have dementia
(biomakers including blood, CSF and neuroimaging), genetic and cognitive/behavioural/clinical scoring.
Human post-mortem and brain biopsy studies.
Invertebrate animal models.
Induced pluripotent stem cells (iPSC) and brain organoids
.

Why were they not suitable?

Clinical longitudinal studies of people who have dementia, provide correlative data between an individual's genetics, environment, biomarker (blood or cerebrospinal fluid biochemistry, or brain imaging data) and cognitive and behavioural outcomes. These data-sets can be used to generate hypothesis of the molecular and cellular causes of dementia-associated biology but cannot be used to test how specific factors influence dementia outcome.

Human post-mortem and brain biopsy studies comparing people who had dementia (early and late in disease course) with people who did not have dementia, can be used to determine which cellular and molecular changes occurred in the brain. In some cases clinical and biomarker data from life will also be available for these individuals

and this observational data can be used to hypothesis which cellular and molecular changes resulted in clinical disease. However, these samples cannot be used to test all hypotheses.
Invertebrate (fly models) can be used to study the response of neurons to the proteins that misfolded and aggregate in dementia-causing diseases and to screen for genetic modifiers of these processes. However, they do not have complex behavioural and memory biology and thus it is not possible to study the cellular and molecular processes that result in altered executive function or sub-types of memory in dementia. For example, it is not possible to quantify working memory, motivation or apathy in a fly. Flies also lack key immune brain cells (microglia) and a closed circulatory system (veins and arteries) which have key roles in the development of neurodegenerative disease. These aspects of the biology of dementia cannot be modelled in flies.
Similarly, iPSC and brain organoids cannot currently be used to study cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole body physiology.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Current estimates are that we will need a sample size of 10 per sex and genotype for each phenotyping experiment (see example power calculations in P3). In next-generation preclinical mouse models of dementia-causing diseases, which will be typically used in this project some key dementia-relevant phenotypes do not develop until mice are 12-18 months of age. In order to ensure we have sufficient sample size at later-time points it is necessary to account for an expected attrition rates of 25% (this includes culling of mice to avoid the phenotyping of lone-housed because of loss of cage-mates. Rehousing of females will be undertaken but will need to be included as a variable in subsequent analysis).
A typical study will include 4 genotypes of mice, thus (4 (genotypes) x 14 (mice) x 2 (sexes)) =112 mice will be required for each experiment on P3. We anticipate undertaking approximately 25 such experiments per year for every year of this project.

At least 2-rounds of breeding will be required to generate the mice required for the experiments outline in P3 and we anticipate providing other researchers with cohorts of mice matched to those studied in P3 for collaborative phenotyping projects. Thus, in total we estimate we will need to breed 40,000 mice for this project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Power equation calculator will be used for group size calculations for P3 experiments plus attrition rate calculation will also be used to ensure sufficient power is maintained until the end of the longitudinal studies.
The combination of tests in each experiment will be designed to gather the most meaningful data. Tests which can inform each other will be carried out on the same mouse to remove inter-animal variability and increase the power, thereby decreasing the overall sample size and the scientific utility of generated data. This approach will also provide novel scientific insight into the relationship between dementia-relevant phenotypes and help validate new testing approaches such as home-cage assessment. We will also phenotype over the life-span of the animal to improve power and to understand the progression of disease and effect of age.
SOP's have been written and used routinely for previous projects. This standardises the way the data is collected and reduces the variability and therefore the sample size.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Efficient breeding and holding lines as frozen down embryos and sperm will be used to minimise the number of mice being produced for these studies. Genetically modified lines will be sourced from repositories to avoid remaking of lines whenever possible. Any excess stock will be offered to other researchers to minimise wastage. Pilot studies will be undertaken to generate means and standard deviations for work using back-ground strains for which data is not available. Tissues sampled from the animals used in this project will be shared with other researchers and the data produced linked to that generated by the project, to maximise long-term utility.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Some mouse models of diseases that cause dementia frequently develop fits or movement problems. Here we plan to use alternative mouse models that do not develop these problems as much.
Moreover, by collecting data from behaviour tests in mazes at the same time as also monitoring the natural behaviour of the mice in their cages, we will help develop new ways to study the behaviour and learning of mice in their normal environment. In the future we hope that this will make the experiments to understand how mice behave and learn less stressful for them.

Test	Why is this the most refined method?
Ear biopsy	Mice need to be ear clipped for identification and the same piece of tissues is used for genotyping. Genotyping protocols have been optimised to use this very small samples to avoid the need for other samples to be taken.
Induction of expression using of genes.	In most cases this will be done by oral dosing. This is more refined than injection, as transgene whilst it involves restraint there should be no pain. Injection will only be used if oral expression and dosing will not work. These methods can be used to avoid the effect of changed changes in gene expression happening in the young mouse which may stop it growing properly and injection can also reduce the number of mice needed for some experiments.
Tests of behaviour and vision	These involves placing a mouse in an arena or maze and allowing it to explore the area or interact with objects and pictures. Light levels are set so that the mouse can see but are not intended to be anxiety inducing.
Social recognition	Light levels are low to reduce anxiety. In this test another mouse is present in a section of the arena. This mouse is in a small cage to stop any aggression but the scent and sight of it are needed to understand how the mouse behaves to other mice.
Temperature taking	This is done using a rectal probe that should cause no more than transient discomfort. The animal is only lightly restrained and the test is done in the shortest time possible, usually less than one minute.

Light dark box and Elevated plus	This is non-invasive and involves placing a mouse in an arena and allowing it to explore. Light levels are anxiety inducing in some arena of the arena but not others. This is necessary as the test is assessing the preference of the mouse to the darker maze or lighter areas.
ECHO-MRI	This test involves less than one minutes of light restraint whilst body composition is measured. This is more refined that alternative tests which require general anaesthesia and a longer time to gather the data.
Blood sampling	Blood samples are collected to a maximum of 15% total blood volume of the animal, which has shown no adverse effects in previous studies. Samples are taken from the tail vein using a very small cut and mice have local anaesthetic applied to the area twenty minutes before.
Tolerance tests	Blood samples are collected to a maximum of 15% total blood volume. Fasting for this tests is usually 18 hours due to the need to allow animals to use up glucose supplies. However, where possible fasting times will be reduced. This test is more refined than other methods of measuring glucose which require surgery to implant a sampling tube.
Home cage monitoring	This is non-invasive and measured in the home cage. After initial insertion of a microchip this test involves no further pain, suffering or distress to the mouse.
Auditory brainstem	This test is done under general anaesthetic due to the extremely sensitive nature of response the test. Mice are monitored at all times through a window.
Touch-screen tests	In this test the mouse learns a series of pictures on a computer screen and that it receives a food-reward for correct answers. It can be used to test motivation and is less stressful for mice than other motivation tests, such as seeing how long the mouse will swim for before giving-up.

Why can’t you use animals that are less sentient?

Here we want to understand how the diseases that leads to dementia change the bodies biology and which changes cause which dementia symptoms.
Invertebrate (fly models) can be used to study the response of neurons (brain cells) to the proteins that build-up in the brain in dementia. They can also be used to find out which genes are important to this. However, flies have simple behaviour and learning and thus it is not possible to study all dementia symptoms in them. For example humans and mice are able to make plans and think ahead to complete a task and experience emotions that can affect how they behave (such as feeling unmotivated). These types of memory and behaviour are affected in people who have dementia and cannot be studied in a fly. Flies also lack immune brain cells (microglia) which are known to have a very important role in the diseases that cause dementia. The cardiovascular system (heart and blood vessels) of flies significantly differs from that in mammals like humans and mice (being open without veins or arteries). Thus the effect of heart health on dementia cannot be readily studied in a fly but requires a mammal such as a mouse.
This work needs to be undertaken in an adult with an intact nervous system without anaesthesia because we need to measure how disease-causing changes in the brain affect how the animals behave.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Here we aim to collect a wide range of phenotyping data to understand dementia-causing diseases. These data

will also be used to understand the biology that underpins changes to mouse behaviour and will also be used to monitor and improve animal welfare, drawing on a welfare improvements technology development pipeline.
For all tests it is important that the animal has no additional stress, therefore mice are handled calmly and habituated to testing rooms as well as arenas if possible.
For all tests mice are only housed in modified cages or arenas for the minimum time needed to gather meaningful data. Mice undergoing phenotyping tests have increased monitoring and are removed from tests if they appear to be suffering from an adverse stress reaction, or other unexpected adverse effects of the phenotyping tests.
Monitoring of mouse behaviour in the home cage and development of tools to analysis these data will be run in parallel with task-based behaviour tests, with the long-term goal of refining mouse behavioural testing particular for mouse models of dementia-causing diseases.
All surgery will be undertaken in full compliance with Laboratory Animal Science Association aseptic technique guidance to minimise infection risk.
Mice which have had anaesthesia have extra monitoring until fully recovered and extra checks when back in the holding rooms. When general anaesthetics are necessary, the combinations with least adverse effects will be used, for example for all tests inhalation anaesthetics will be used, with the exceptions of ABR and OCT which logistically cannot be carried out with the mouse on a face mask. Pain from tail bleeds is reduced by using local anaesthesia.
Experiments will be designed to balance the overall experience of the mouse and the number of type of tests any one animal against the value of a full understanding of the biology of individual animals and why phenotypes are altered in order to maximise utility of the research data obtained.
Technical refinements will be developed throughout the life-time of the project and disseminated to other researchers at the Institute.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Routes and volumes for administration of substances are taken from Laboratory Animal Science Association good practice guidelines: administration of substances 1998 (http://www.procedureswithcare.org.uk/lasa_administration.pdf).
Surgery will be undertaken as per the Laboratory Animal Science Association Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2017 (http://www.lasa.co.uk/wpcontent/uploads/2017/04/Aseptic-surgery-final.pdf)
The animal house has full AAALAC and ISO9001-2015 accreditation. To conform to these standards we must ensure a high level of quality control on all fronts including husbandry, phenotyping and administrative processes. Standard operation procedures for most tests have been generated using data and expertise from multiple animal houses and can be found at https://www.mousephenotype.org/impress ARRIVE and PREPARE guidelines will be followed at all times.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I will attend the NC3R annual conference, I will also attend international meetings focusing on animal models of dementia causing disease and monthly webinars run by the MODEL-AD consortium. In addition I will attend local 3Rs seminars and events.

Home Office

NON-TECHNICAL SUMMARY

224. Using zebrafish models to understand impaired wound healing

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Inflammation, Angiogenesis, Wound healing, Diabetes

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand the mechanisms underlying how inflammation and blood vessel growth interact to drive normal wound healing, and how these go awry to result in compromised tissue repair, particularly in the context of diabetes.
Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Over 1 million compromised wounds are treated annually in the UK, resulting in increased morbidity and mortality rates, incurring significant reduction in quality of life, and are a huge burden on health services. Diabetic wounds account for a major subset of compromised wounds, with 15-25% of nearly 500 million diabetes sufferers worldwide developing non-healing wounds such as foot ulcers, the most extreme cases requiring amputation. Crucially, compromised wounds often prove unresponsive to current clinical approaches, with wound recurrence rates of up to 70%. Compromised wounds broadly share the same causative mechanisms of dysregulated wound inflammation and angiogenesis, however how these processes interface and how they go awry remains poorly understood. Improved mechanistic understanding of these processes is the most likely path to ameliorating the impaired healing of compromised wounds.

What outputs do you think you will see at the end of this project?

This project will deliver novel insight into how processes underlying normal wound healing go awry to result in compromised wounds. Specifically, we will use the zebrafish to identify and rapidly screen genes whose expression is modulated in compromised and diabetic wounds, but whose function in tissue repair remains unclear after decades of clinical and rodent research.
Beyond providing vital improvements to our understanding of this basic biology in the short term, these zebrafish models will have longer term benefits as a platform for drug and biomaterial discovery and screening, identifying how these defective processes might be rescued to improve impaired tissue healing. Ultimately, this will result in the generation of new products (e.g. dressings, topical preparations) that can be propelled towards the clinic. Both the basic and translational aspects of this project will result in numerous high impact publications, which in turn will allow this important information to be disseminated to others through conferences, press releases and public engagement events.

Who or what will benefit from these outputs, and how?

This project addresses the important question of how processes underlying normal wound healing go awry to result in compromised wounds, particularly in the context of diabetes, and how these can be corrected to re-establish tissue repair. The mechanistic insight and therapeutic leads generated by this research will have a critical impact in understanding and treatment of compromised wounds – 70% of which present as ‘hard to heal’ and respond poorly to existing therapies – and may have more broad ranging impacts in treatment of other non-healing tissues. In the short term, the main beneficiaries of this work will be academics interested in tissue repair, immune cells and inflammation, blood vessel development and disorders as well as bioengineering, who will discover the findings of this work via conferences, publications etc. and with whom collaborations will be established to expand upon this work. Furthermore, this project will generate highly trained researchers offering unique interdisciplinary skills, providing transferable skills to both the researchers involved and potentially other non-academic beneficiaries. There are numerous longer term beneficiaries from this work, including:
1) Clinicians/patients demanding more effective therapeutics for compromised wounds, which are a major cause of morbidity and mortality that currently lack effective treatments. Improving and accelerating the development of technologies that treat compromised wounds towards the clinic will greatly benefit patients suffering from these disorders. This project will therefore impact on quality of life, health and well-being, as efficient wound healing is a prerequisite for healthy ageing. Collaboration with clinicians will feed back into the design process, to establish the best possible technologies and techniques for clinical use.

2) Pharmaceutical companies wishing to discover treatments for tissue repair disorders. The models established in this proposal will be attractive resources for the pharmaceutical industry, allowing rapid drug development and testing of existing small molecules or re-purposing of existing chemical compounds for wound

healing functions. Protection of IP for these targets as they are discovered will bring significant economic gains to the UK economy.

3) Industrial biotechnology partners wishing to improve existing wound healing technology and techniques. Collaboration with these partners will permit mechanistic testing of their technologies, allowing them to improve the functioning and marketability of their products. This leads to longer-term commercial opportunities to develop technology at a higher throughput level. Industry investment in this programme and IP sharing arrangements will ensure mutual benefit from emerging knowledge for all parties.

4) NHS and health care policy makers facing tight budgets and difficult decisions as to where resources such as staff and equipment should be allocated to deal with a large range of clinical needs. Care for sufferers of compromised wounds annually consumes more than £5 billion and over 40 million doctor and nurse visits, as this condition requires careful daily management and care for weeks to years to resolve.

How will you look to maximise the outputs of this work?

To ensure other researchers benefit from our work, academic audiences will be reached by journal publications, as well as presentations at conferences and seminar series. Collaborations with other interested researchers will be actively pursued. Where practicable, we will also aim to make the results and microscopy data available via a custom built website (developed with support from a specialist academic web design team), from both successful and unsuccessful experiments.
We will use ZFIN and EuFishBioMed to engage and inform industrial parties interested in zebrafish studies of the models being established and research direction undertaken. We will also visit patient support groups, charities and clinics with high numbers of patients that suffer from compromised wounds, e.g. diabetes clinics, to engage these patients and the health care professionals that treat them, communicating the cutting edge research into wound healing biology that is being performed. **Species and numbers of animals expected to be used**

- Zebra fish: 19500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using zebrafish as they are the best vertebrate model organism for rapid identification and mechanistic screening of disease associated genes, as well as visualisation of how various tissues interact with each other in vivo and in real time. We have specifically focused most of our study around the larvae, as this is the maximally translucent stage of the fish while having most of the important tissue types that are relevant in human wound healing. We will also expand some of our work to adult fish, which are not as easy to image but present robust models of Diabetes that have an increased tissue complexity approaching that seen in human tissues.

Typically, what will be done to an animal used in your project?

The focus of this project is to generate animals that have mutations in genes and pathways that are impaired in Diabetes, to identify how these genes effect inflammation, blood vessel growth and wound repair, and how this might be rescued. As such, wild type and mutant fish will undergo one or a combination of wounding, biomaterial implantation and exposure to bioactive molecules that modulate inflammation/blood vessel growth. As an example, a common larval investigation would entail making a small wound with a 30G needle at 4 days post fertilisation

(dpf), with subsequent treatment with anti-inflammatory drugs e.g. Hydrocortisone to modulate inflammation over the course of wound healing (up to 14dpf). This drug treatment may be delivered either systemically by IV injection/addition to the fish water, or locally by injection of beads functionalised with the drug. These fish could then be imaged at various timepoints post injury to establish the progression of the entire healing response within the same group, e.g. imaging at 2 days post injury (6 dpf) to observe blood vessel sprouting, 5 days post injury (9 dpf) to observe blood vessel maturation and 8 days post injury (12 dpf) to observe blood vessel remodelling and wound resolution. Alternatively, these fish may undergo long term timelapse to capture detailed cell interactions at particular points of the wound healing response. In any case, any fish that undergoes experimentation on this protocol will be humanely killed once experimentation is completed.

What are the expected impacts and/or adverse effects for the animals during your project?

We expect that some short term pain will result from our wounding/implantation experiments, categorising these protocols as moderate severity. Our Diabetes models may also be expected to result in weight gain and other adverse effects related to this disease (e.g. high cholesterol, eye and kidney damage). For larval experiments, we will end both wounding and Diabetes induction experiments no later than 14 dpf (10 days post injury). For adult fish, we will treat them with our procedures to induce Diabetes for no longer than 8 weeks, and wounding/implantation procedures on adult fish will be followed for no longer than 4 weeks.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most of the animals included in this project will not suffer more than mild severity, and this is largely categorised as such because they will be genetically altered (for example, many of these will express fluorescent molecules that label specific cells or tissues but are otherwise not expected to cause any harm to the fish). Approximately 30% of fish will be classified as 'moderate', due to the use of wounding/implantation surgical procedures that potentially cause short term moderate pain, or due to these fish being Diabetic and therefore potentially suffering moderate impairment to their well-being.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Wound healing is a complex process requiring many cell types, mediators and interaction between these. As such, an in vivo model is required. Using the zebrafish, we are able to efficiently perform genetic manipulation and rapid mechanistic screening of the roles of these genes in wound healing. In vitro models can be a useful reductionist approach to unpick certain aspects of the interactions between immune and endothelial cells, but they lack the context of the wound environment that is crucial to improve our knowledge of how inflammation and angiogenesis interact.

Which non-animal alternatives did you consider for use in this project?

To complement our fish studies, we have previously used human macrophage-endothelial cell coculture approaches to explore the function of specific interactions that had been initially identified in the in vivo fish wound assay. We will continue to use this approach to validate our fish findings, thereby refining the fish models we use for further screening and development of clinically interventions and reducing fish numbers used in the long term.

Why were they not suitable?

Our co-culture assay is suitable for dissecting specific mechanisms of interest, identifying how conserved these interactions are and validating targets identified in the zebrafish model. However, the lack of in vivo context means that this approach is not suitable for the initial upstream identification and screening of these genetic targets.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Estimated numbers of fish used in each protocol is based upon detailed project planning undertaken within my lab (using previous experience in experiments needed), as well as between my lab and our aquarium team (using combined experience in breeding and maintenance of fish). This experimental design takes into account factors such as number of fish required to safely maintain breeding stocks, breeding new generations of fish at an appropriate age to prevent welfare issues that can arise in ageing populations, and generation of sufficient alleles of new mutants for robust genotype/phenotype correlations.
Protocol 1 - 10000 adult fish over 5 years, from a range of backgrounds, both wild-type and transgenic.
2000 of these fish will be used for Protocol 3 tissue repair studies, 500 in Protocol 4 and 150 in Protocol 5. The rest of these adults undergo no procedure other than breeding and occasionally fin clipping or gentle gamete expression.
Protocol 2 - 8000 adult transgenic/mutant fish over 5 years. For most transgenic and mutant lines, we raise approximately 100 embryos to adulthood to identify a minimum working stock of positive carriers/founders. We will make a maximum of 16 lines per year for 5 years, giving a total of 8,000 adults. 1500 of these fish will be used in Protocol 3.
Protocol 3 – limited to 4000 adult fish over 5 years, and only where experiments cannot be performed on younger animals. As part of good laboratory practice, we will write a study plan for each experiment involving regulated procedures including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, details of the experimental material, and the size of the experiment (number of groups, numbers of animals/group); and an outline of the method of analysis of the results.
Protocol 4 - 2000 adult fish over 5 years, either diabetes mutants or chemical induced diabetes fish. These adults are the key colonies to this project, providing the necessary animals to examine the specific disease context of interest. Where possible, we will use fin clipping (or ZEG) at <5dpf to identify founders and reduce the numbers of embryos raised. 500 of these fish will be used in Protocol 3 tissue repair studies, and 50 in Protocol 5.
Protocol 5 – 200 fish for manual expression of gametes. Occasionally, female zebrafish become egg bound and no longer lay eggs. In order to reduce the number of fish used, these fish can be returned to full reproductive health by gentle expression of gametes. We anticipate we will need to perform 200 such procedures during the course of this licence, reducing fish numbers in other parts of the licence by this amount.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Where applicable we will use the NC3R's Experimental Design Assistant to consult on sample sizes for individual experiments to ensure only the minimum number of animals required is used. In addition, we have sufficient pilot data from all studies to perform a priori power calculations to calculate group sizes. We will not initially exceed the predicted group sizes required to detect a 25% difference with 80% power (alpha of 0.05 as per convention), allowing us to detect relatively small effect sizes associated with a biological process likely to have a large amount of variability (wound healing). We will repeat experiments with increased group sizes only if greater power is required, if the variance is greater than in pilot studies, or where the biological effect to be detected is less than 25%. Where we obtain data over time from the same animals, we will use statistics appropriate to repeated

measures. The use of the same animals over time greatly reduces the animal number required (compared with groups of animals sacrificed at each timepoint as occurs in many mammalian studies).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

As indicated, we have used pilot studies to assist in the experimental design phase to reduce estimated numbers of fish required – we intend to continue pilot programs to inform new directions, to be constantly re-evaluating our data and to seek statistical advice to optimise animal numbers. Where feasible, early genotyping using a zebrafish embryonic genotyper system will reduce the number of larvae raised as we will only raise those of the required genotypes. We have also been early adopters of techniques to increase transgene incorporation and of transient CRISPR approaches to reduce the need for generating mutant lines. Where these approaches are suitable, we will use them to reduce the number of embryos that require raising to identify founders.
Where necessary, gametes are expressed either to maintain fertility or to allow in vitro fertilisation, and numbers are determined by the requirements of maintaining fertility and preserving lines. This procedure allows reduction of numbers required in other procedures, by preventing the sacrifice of overburdened females and by preventing the keeping of live stocks where a frozen stock would suffice.
We will also utilise individual Study Plans for experiments using protected animals. These provide the researcher the opportunity to determine:
1) They have authority on the PPL to perform the experiment (i.e. the purpose of the experiment fits with one of the objectives detailed in the project plan and that there is clear authority on the protocol to perform the procedure). In addition, that they have the relevant skills detailed on their PIL and training and competency record.

2) An opportunity to determine how many animals are required per group (including controls) to answer the question.

3) Write a clear description of what procedures each animal will receive, e.g. i.p. injection of drug X at day 1, followed by imaging analysis under terminal anaesthesia on day 6) Write down any adverse effects that may be associated with the procedures and describe clear humane endpoints.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Zebrafish have a number of advantages for these studies, including optical transparency, genetic tractability and extensive genomic resources. The use of reporter lines in zebrafish allows in vivo visualisation of cell behaviour and morphology, which is required in my project to analyse immune cells and blood vessel interactions. Moreover, the ability to rapidly screen gene functions by newly developed CRISPR technology allows for the identification of key genes and pathways that elude higher vertebrate models. Importantly, zebrafish models of diabetes recapitulate many of the pathological aspects seen in humans, and the immune system and endothelial cells of zebrafish shares significant homology with human. This makes the zebrafish a relevant model to study the interactions between these populations, and how these go awry in diabetes. Furthermore, the zebrafish is the organism with the lowest neurophysiological sensitivity that is suitable to study the interactions between the innate immune system and blood vessels during tissue repair to address our proposed aims.
The methods for generating mutants and transgenics are optimised to minimise numbers and adverse effects are minimal. Fin clipping and gamete expression methods rarely involve inducing any discomfort, but for all manipulative procedures animals are suitably anaesthetised (primarily for restraint). Anaesthetic is delivered by immersion (so no invasive instrumentation is required) using an anaesthetic protocol suitable for the procedure and following best practice.

Why can’t you use animals that are less sentient?

This project focuses on primarily using zebrafish as a platform for studying wound healing, as this is the lowest vertebrate model in common use that has most of the conserved structures compared to human tissue (skin, muscle, nerve cells, immune cells and blood vessels etc). Less sentient animals e.g. nematodes lack many of the structures and much of the genetic complexity that would make such studies clinically relevant. Where possible, we also focus on using fish at the earliest developmental stage when all necessary cells and structures are present, with most assays being performed at 4 days post fertilisation (prior to the age of protection).

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Where feasible, various recently developed refinements are used: (i) the use of a zebrafish embryonic genotyper system, (ii) swabbing instead of fin clipping and (iii) the use of a DanioScope (or similar) for observing early phenotypes.
Genotyping adults requires fin clipping under anaesthesia but we have also included skin swabbing which does not require anaesthesia. This technique will be tested and upon robust and consistent genotyping results will be adopted to genotype all adult fish.
Where possible, potential therapeutic interventions (e.g. biomaterial implantation, exposure to bioactive molecules) will be assayed before 5.2dpf and only animals with signs of recovery will be raised past protected age. All animals involved in wounding, intervention or diabetes protocols will be closely monitored for healthy swimming, feeding and socialising behaviour. Pilot data suggests that wounding and treatment groups only require relatively small numbers (5 to 10 animals). To increase animal health and wellbeing if stressed or kept in lower density, environmental enrichment (e.g. laminated gravels) may be placed in or under the tanks.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

ARRIVE guidelines, and specific research guidance covering responsible use of animals in bioscience as made available by organisations such as UKRI.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The zebrafish community in general, and the community at the REDACTED in particular, is constantly looking for ways to advance and promote the 3Rs. There are numerous bodies that disseminate these advances, from journals (e.g. Zebrafish) to local and international conferences, which we will endeavour to attend and engage with. Moreover, we will routinely monitor resource websites such as those run by NC3R and research council bodies such as UKRI for new tools and guidance, as well as engaging with the NC3R's Regional Programme Manager. New advances will be implemented where possible and with the assistance of the experience technical staff at the REDACTED aquarium, as we have already demonstrated in this project application.

Home Office

NON-TECHNICAL SUMMARY

225. Viral tumourigenesis and inflammation

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

cancer, virus, therapy

Animal types	Life stages
Mice	adult, juvenile, pregnant, neonate, embryo, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Epstein-Barr virus (EBV) is a common human virus associated with several forms of cancer. The long term aim of the project is to understand viral disease mechanisms and enable the design and testing of therapeutic strategies and regimens against virus-associated cancer. The aims over the next 5 years include: to determine factors that influence the chronic inflammation that arises in several of these cancers; to discover the mechanisms by which specific viral proteins contribute to cancer development; to test candidate therapeutic treatments for efficacy.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

EBV is linked to approximately 200,000 cancer cases per year (worldwide) and is classified by the International Agency for Research on Cancer as a group 1 carcinogen. Burkitt's lymphoma (BL, which is a blood cancer) and nasopharyngeal carcinoma (NPC, which is a cancer that forms at the back of the throat, in the nasopharynx region) are two types of cancer most closely linked to EBV and together these present a significant world health disease burden. In order to gain an in depth understanding of the role played by the virus in these diseases and from there, to develop and test specific therapeutic regimens that have minimal side effects, it is necessary to have scientific, pre-clinical models of disease that faithfully recapitulate the viral component. From the safety of the laboratory, such models permit in depth molecular analyses of disease processes, providing knowledge that forms a platform for therapeutic design and disease intervention. The pre-clinical models then provide the early stage testing ground for novel treatments, that necessarily precede clinical trials.

What outputs do you think you will see at the end of this project?

By the end of this project I anticipate several new scientific publications reporting on the scientific information learnt as a consequence of these studies. I expect this to include new information regarding the role of certain cellular proteins in carcinogeneisis and inflammation, a deeper understanding of EBV associated tumourigenesis, and assessment of new treatment modalities for EBV-associated cancer. This new scientific information will contribute to the ongoing global efforts to treat and cure EBV associated disease. The study of inflammation will also shed light on chronic inflammatory diseases in general. This new information will be disseminated in the form of peer-reviewed scientific publications, but also through national and international conferences and other scientific forums for information exchange (such as seminars and lectures).

Who or what will benefit from these outputs, and how?

The scientific community will benefit in the short term from the new information gained. The knowledge will contribute to a greater understanding of EBV-associated disease processes and potential treatment routes. The cumulative scientific knowledge on this topic will benefit world health in the longer run, in directing the development of new treatments for these diseases. Scientific knowledge is arrived at in small bite-sized chunks, with multiple labs contributing to the information. It is the cumulative knowledge that can then be put to greater clinical use.

How will you look to maximise the outputs of this work?

In the past 5 years I have collaborated with labs across Europe and the USA and together we have published our work in peer reviewed scientific journals. These collaborations were initiated by collegiate discussion at scientific conferences. Likewise, over the next 5 years I will disseminate data from my lab through seminars and presentations at scientific conferences and embark upon new collaborations. In addition I will publish our data, both work that supports the hypothesis under test and data that might refute a hypothesis, as both are valuable in the research setting. I will contribute to public outreach events to disseminate new knowledge in the area of this work.

Species and numbers of animals expected to be used

- Mice: 4,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the only animal to be used in this project for several reasons. First, domestic mice can be kept easily in cages, they eat well and are happy to breed and appear to be unstressed in captivity. Second, their whole genome has been sequenced and they are highly genetically similar to humans, with the same genes and molecular processes involved in disease pathways as humans. Also, mice have been commensal with humans for thousands of years and as a consequence, their immune system has evolved along very similar lines to humans. Thus functions at the molecular, genetic, cellular and whole tissue levels (such as immune responses) are likely to be very similar between mouse and man, if not identical. Therefore, mice provide excellent models of human disease, to both understand disease processes and test potential treatments. This is especially true for cancer and immune system disorders, such as chronic inflammation. Finally, due to these advantages, there are hundreds of genetically altered mouse disease models available in the scientific community, providing a wealth of data that allows mass integration of this vast knowledge and thus deeper understanding.

Typically, what will be done to an animal used in your project?

Many of the mice in the project carry one or more of the viral genes under study within their genome. This leads the mice to display part of the disease characteristics that is seen in humans and allows the study of that characteristic. For the most part, the progression of the disease characteristic is monitored visually and finally tissues will be collected to permit an analysis of samples. Some mice will be treated and this will involve the application of a substance (for example a drug under trial), which may be injected, applied topically, or in the drinking water. How this affects the disease characteristic is then examined. At times, a blood sample might be taken from the mice. Some mice will undergo painless imaging under anaesthesia (so that they remain still), to quantify disease features and progression and this is also used when testing treatments, to determine how effective the treatments are.

What are the expected impacts and/or adverse effects for the animals during your project?

This project aims to understand processes in cancer and inflammation. As such, some mice on the project will experience chronic inflammation of the skin, not unlike psoriasis, from mild to more severe. This will be a persistent characteristic in some mice. Some mice will be expected to develop tumours, either carcinomas (typically on the skin) or lymphoma. If the tumour causes the mouse any suffering, the mouse will be humanely killed directly.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately half of the mice on the project will show some disease characteristic from mild inflammation of the skin, to tumour formation.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The complex interplay between the immune system and cancer cannot be faithfully recapitulated in the test tube or in cell culture systems. Similarly, the diverse action and consequences of drug or other treatments in the body, cannot be determined in culture experiments alone. Moreover, all new therapeutic regimes and medicines benefit from being tested in an animal model system before translation to human trials. As such, non-animal alternatives for the described objectives either do not currently exist or don’t fulfil the objective.

Which non-animal alternatives did you consider for use in this project?

Tissue culture and laboratory alternatives (such as molecular and biochemical analyses) are considered and used, wherever possible, to contribute to the study. These approaches are used before animal studies begin to provide as much information as possible as a platform for the study in animals.
Equally, such methods are used after the animal studies to follow up on specific aspects of the findings.
Why were they not suitable?

At present, tissue culture methods do not faithfully recapitulated complex physiological systems, such as the immune system, or the complexity of organ function. In part, this is because these systems are highly complex and interactive and are not fully understood, but also in part because technology has not yet advanced to a stage where the complexity in the body can be replicated in a plastic dish. Similarly, biochemical and molecular experiments can allow determination of the fine detail, but fall short of predicting the greater connectivity within the mammalian body.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers have been estimated based on considerable previous experience. Furthermore, the average annual animal use on the preceding project, over the last 5 years has been between 200 and 400. A similar plan is proposed over the next 5 years, but there is possibility for the expansion of these studies (by no more than double). As such, 800 per year represents a maximum.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

It is important that studies are conducted to produce reliable data subject to statistical scrutiny. In all protocols the numbers of mice required to generate a statistically significant and/or otherwise meaningful result are considered carefully in advance of the experiment (using both considerable experience and design tools such as that available on the NC3R site) and numbers of mice used do not exceed this.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

At the end of study, tissue samples are collected from all mice and used for experimental assays in the lab and/or shared with other labs that use tissues in their studies, thus optimising the use. The use of in vivo imaging for certain studies (particularly time courses) reduces the numbers of mice required on study compared to conventional assays. The use of pilot studies to enable reliable calculations for subsequent larger studies is used as standard.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice provide an excellent model for human disease, particularly diseases of the immune system and cancer, for

several reasons. They are highly genetically similar to humans, suffer from the same range of diseases and have an almost identical immune system to humans. As such, determining gene function and immune responses in mice has proved to be hugely informative with regard to human disease. In this project we will use mice with genetically altered genomes alongside normal mice to address the aims described. We will use well established reagents and protocols to study the mice. In all protocols, the procedures which cause least pain and distress in achieving the objective are followed. In all cases the mice will be closely monitored and any mouse showing signs of distress during the procedure will be humanely killed.

Why can't you use animals that are less sentient?

The aims of this project concern disease modelling and treatment of cancer. There is an intricate interplay between cancer cells and immune system cells and this factor is especially important to this study. The immune system of mammals is substantially different to other animals, especially those less sentient and the study is simply not possible in non-mammalian species. The immune system of the mouse is highly similar to humans, which makes it an ideal animal model for such studies, indeed, in evolutionary terms (and therefore also genetical terms) rodents are the closest animal group to primates.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In all protocols, the procedures which cause least pain and distress in achieving the objective are followed. As refinements to these procedures become available (either published or advised), these will be implemented where possible. We are working to refine the sampling methods for genetic screening. We have developed and are optimising a system using fluorescent factors to track tumour development by imaging. This provides a more robust method to assess the efficacy of therapeutic treatments.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The institution has established best practice forums and provides such guidance and this is based upon veterinary advice and up to date published information. In addition, information provided in "Guidelines for the welfare and use of animals in cancer research" Workman et al. (2010, Br. J. Cancer 102:1555-1577), ASPA "code of good practice" website and NC3Rs website, will be followed.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Advances in the 3Rs are reported in the scientific literature, on the NC3Rs website and through institutional bulletins and practices. Such advances will be implemented alongside institutional and veterinary guidelines, practice and advice.

Home Office

NON-TECHNICAL SUMMARY

226. WILD MAMMAL POPULATION STRUCTURE

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (d) Protection of the natural environment in the interests of the health or welfare of man or animals. (e)
- Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work. **Key words**

bats, dormice, wildlife conservation

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The work conducted under this licence is intended to inform directly the conservation of dormice and bats. It fills key knowledge gaps identified by decision-makers including Natural England and Local Authorities that are currently barriers to effective population management. The two main aims of the work are to

1. Develop robust assessments of population size and trends;

2. Assess of the impact of habitat loss and fragmentation on the conservation status of the target species.

These areas are repeatedly highlighted as research priorities for the mammals studied in this project (e.g. Detra 2011; State of Nature Report 2013; JNCC 2013; Sutherland et al. 2006). Bats and dormice are of high conservation concern across Europe due to dramatic historical declines in their distribution and abundance (Habitats &c Species Regulations 1992). The information generated by this project will not only addresses ecological questions of scientific interest, but will also contribute to the ability of the UK's competent authorities to discharge their statutory duties to monitor the population size and conservation status of the study species.
The objectives are:

1. improve estimates of the population size and structure of the target species.

2. validate survey methods routinely used by professional ecologists, contributing to the development of better methods for monitoring changes in conservation status over time.

3. provide quantitative evidence on the effects of different forms of habitat fragmentation on population structure, social networks, animal dispersal and genetic interchange.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The work will provide a better evidence base for the conservation management of the species. It will also help stakeholders discharge their statutory requirement to monitor and report on the size of British populations, their change over time, and the primary threats facing the target species.
Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

This project will focus on dormice and bats. It will involve approximately 100 dormice which will be radiocollared and also microchipped (using the same technology used for identifying pet animals). Samples will be taken from approximately 500 bats for analysis of their genetic relatedness and place of origin.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The severity of the procedures is low.

The marking procedures (PIT tagging and ringing) and fur clipping on their own would fall outside the remit of the Scientific Procedures Act.

The main risk is that fixed radiocollars are carried by the animal for life (<5%) and present a small risk of entanglement (<2%). Therefore a range of different approaches are being deployed to try to ensure that the collars are removed at the end of the monitoring period. There are small risks of abrasion (<2%) from the collar which are being addressed through the use of appropriate materials and careful monitoring of recaptured animals. There is a small risk of bleeding or infection from the wing puncture site and site of insertion of the PIT tag (<1%). These risks will be minimised by the use of new sterile equipment and careful technique.

All the animals will be released back into the wild at the point of capture.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

This project specifically investigates wild animals in their natural environment so non-animal alternatives are not possible. The data will help to improve non-invasive approaches such as the use of faecal samples for genetic monitoring

Reduction

Explain how you will assure the use of minimum numbers of animals.

The principal investigator has in-depth knowledge of statistical design, and will keep the proposed sample sizes under constant review during the project. The sample sizes will also be agreed by Natural England which has statutory responsibility for safeguarding the conservation status of the species concerned.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The study aims to gather species-specific information in order to inform dormouse and bat conservation. However they are also good models with which to assess the effect of habitat fragmentation.
Radiocollars have previously been used with a range of small mammals to provide detailed information which cannot be gathered in any other way. Steps are being taken to minimise the likelihood of any adverse effect including ensuring that collars never exceed 10% of body weight, making exhaustive attempts to retrieve all animals in order to remove collars; and use of appropriate materials and fitting techniques to minimise the chance of abrasion. Wing punch samples are generally considered the best means of obtaining high quality DNA samples and are rarely associated with adverse effects. The welfare costs will be minimised through the use of sterile equipment and appropriate technique.

Home Office

NON-TECHNICAL SUMMARY

227. Wound healing and Ano rectal Fistula

Project duration

0 years 6 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants.

Key words

Fistula, ano-rectum, therapy, stem cells, collagen

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Current treatment options for ano-rectal fistulae (an abnormal connection between the anus/rectum and outer skin) are inadequate resulting in poor and variable healing rates. Patients experience considerable pain and poor quality of life. Having previously investigated why these fistulas don't heal we have developed a collagen paste. Our goal is to use this paste to heal the fistula by replacing the lost collagen framework within the fistula tract with one supplied by the paste. The paste can also be combined (optional) with antibiotics or the patient's own stem cells to speed up the healing process.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration

of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Current treatments are either surgical or non-surgical, the former can result in incontinence (lack of voluntary control over urination and defecation) and the latter in variable healing (it may or may not work in each patient). The benefit from our approach is that it will not damage the surrounding tissue and therefore, even in the unlikely event it doesn't work, it will cause no harm, unlike surgical treatments. It has been specifically developed to repair the damaged tissue and can be used as vehicle for the delivery of either drugs or cells to promote healing. The scientific knowledge gained would support the concept that in order to promote long term healing, the underlying structure of the tissue is crucial and where possible we should be aiming to replace like for like.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Our chosen animal is the pig due to it close resemblance in anatomy and physiology to humans. Over the course of the licence approx. 10 pigs will be used

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The severity of the study is moderate because the experiments require all animals to undergo a surgical procedure, possible adverse effects may include pain (moderate).
No adverse effects are expected from the surgical procedure itself. In our experience, animals tolerate these procedures very well with no adverse effects to their health and welfare.
There is a very remote risk of bleeding and infection post surgery, or loss of the indwelling setons (this is a thin silicon thread which is placed into the fistula tract to allow it drain and prevent the accumulation of pus). All of these incidences will be closely monitored for and treated if necessary under veterinary instructions and any changes that significantly impact on the health and welfare of the animal would define a humane endpoint and result in the animal being humanely killed.
No adverse effects are expected from the use of immuno-suppression (drugs that prevent rejection) since we have successfully used this in previous studies. Since a full depth analysis of the fistula tract and surrounding tissue will be essential, all animals will be humanely killed at the end of the experimental period.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

An ano rectal fistula is a painful and debilitating disease in humans; treatment options include either surgical intervention with the possibility of permanent loss of voluntary control over urination and defecation or non-surgical treatment with unreliable healing rates. Ano-rectal fistulae created in pigs are unlikely to be as debilitating because unlike humans who sit on their "bottom" thereby placing pressure on the ano rectal fistula, pigs predominantly lie on their belly or on their side resulting in less pressure and soreness on the outer surface of the ano-rectal fistula. We have developed a novel collagen paste to fill the fistula track and replace the damaged tissue. The paste has been tested in the lab and in lower sentient animals (i.e. rats) to ensure it is safe (i.e. non-toxic) and cell friendly. We are now at the stage where we need to test the paste for its intended clinical application in a model which closely mimics the human with respect to ano rectal anatomy and function.

Reduction

Explain how you will assure the use of minimum numbers of animals.

To date all the necessary experimentation surrounding the development of the paste has been completed and tested in rodents, removing the need to test the paste in large animals. Additionally, a separate study investigating the paste for skin wounds is underway allowing for considerable knowledge to be gained on how the paste performs. This will further reduce the number of animals required in this study. Typically, for comparison of three modes of treatment requiring replicates of 6 of each treatment a maximum of 6 animals would be used as this is calculated to provide robust statistical data during subsequent analysis.
From previous experience, we know we can increase the number of fistulae per animal (up to 3) with no additional cumulative adverse effect, thereby reducing the number animals in each experimental arm.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Our chosen animal is the pig; this animal has a diet similar to humans and hence it anatomy around the rectum and anus closely matches making it an ideal model, including similarities such as e.g. little hair, dermal and epidemal structure, musculature, vasculature and healing properties. Additionally, this model allows for the creation of fistulas of comparable size and potentially similar complexity. Whilst this is the best model, we have been able to develop it is recognised that the pig is quadrupedal (stands on 4 legs) while man is bipedal (stands on 2 legs) and we have accounted for this in the way we analyse our results.
Furthermore, although animals may need to be singly housed for a shorter period as possible after surgery to prevent damage to the wound area by pen mates, our accommodation provides enrichment in the way of toys (e.g. balls and other objects to encourage rooting behaviour) and allows visual, auditory and scent contact with other animals of the same species.
Also by pre-acclimatising the animal, to single housing prior to the initial surgery, any adverse effects can be minimised; we have not observed any obvious adverse effects when using this technique.
Animals welfare will be closely monitored by the NACWO who can be supported by the NVS if required; animals will be placed on a loose diet to ensure a soft stool and reduce the risk of constipation prior to and after the creation of the fistula to help with bowel movement.

Home Office

NON-TECHNICAL SUMMARY

228. ZEBRAFISH MODELS OF CARDIOVASCULAR DEVELOPMENT & DISEASE

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

No answer provided

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We seek to understand the biological mechanisms responsible for development of the cardiovascular system and which go wrong to cause cardiovascular disease. We do this by studying how the heart and blood vessels develop in zebrafish, and by measuring the effect of drug treatment, genetic manipulation or injury on the cardiovascular system.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cardiovascular disease is the leading cause of death worldwide. Despite much progress, we still do not understand in detail how the cardiovascular system develops and how genetic or other disturbance leads to disease. Our work will discover new mechanisms of cardiovascular development and examine the effect of drug and other treatments on zebrafish models of human disease.

What outputs do you think you will see at the end of this project?

This work will provide significant and novel insights into the mechanisms that form the cardiovascular system in vertebrates, and in many cases will provide information about the functions of genes known to, or suspected to, cause human cardiovascular diseases. This will provide the following benefits;

A greater scientific understanding of the basic mechanisms of cardiovascular development
, disseminated through conferences, publications, and other materials including in public engagement events.

A better understanding of the causes of some human cardiovascular diseases
.

Possible discovery of new drugs for scientific study and consideration of clinical assessment
.

A greater awareness of zebrafish models of cardiovascular development and disease
.

These are worthwhile because they will add to the sum of scientific knowledge in a fundamental and clinically important area, they will contribute to reducing, refining, or replacing animal studies in other species, and they may provide scientific insights that could lead to clinical improvements or new therapies.

Who or what will benefit from these outputs, and how?

The immediate beneficiaries will be the scientific community via dissemination of the results of our research. This will also benefit the scientists and doctors trained in my lab, by increasing their understanding of research (including the considerations of animal research) which in their careers they will also disseminate.

In the longer term, the results of our research may identify novel therapeutic strategies that benefit patients and their families. This would also benefit healthcare systems such as the NHS.

How will you look to maximise the outputs of this work?

We have moved to routinely placing all data where possible on publicly available websites such as BioRxiv prior to submission for publication. We frequently take part in public engagement events, speak at conferences, and disseminate our new knowledge in journals and via our websites.

Species and numbers of animals expected to be used

- Zebra fish: 50,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Using zebrafish to study vascular health and disease taking advantage of their transparency in early stages of life. We will also use older fish to study gene function and in particular models of cardiovascular disease where this is necessary because it requires a more developed cardiovascular system than in young embryos.

Typically, what will be done to an animal used in your project?

OMost animals will be bred to generate fish embryos, larvae to study gene function. This may involve manipulation of their genome and/or pharmacolgical intervention to treat or develop phenotypes to understand gene function. We may also use advanced imaging techniques to study the effects of these alterations such as injury to the heart to mimic human myocardial injury, or exposure to glucose as a model of human diabetes.

What are the expected impacts and/or adverse effects for the animals during your project?

Normal husbandry and breeding (protocol 1) is not expected to have any adverse effects. Altering genes that may be required for the heart and circulatory system to develop (protocol 3 and 4) may result in fish that present with heart problems leading to fluid retention (oedema) and/or abnormal swimming behaviours due to reduced heart function. These effects if significant would be likely to be permanent and so would lead to human killing if there is evidence of harm, suffering or adverse effects. Animals that have undergone cardiac injury (protocol 5) will be recovering from surgery and so will experience some post-operative pain which we will address with anaethesia during the procedure and postoperative analgesia. It is expected that the animal will swim normally within hours of the procedure but may swim less for 1-2 days as the wound recovers. Feeding should not be affected for longer than this and if so we would monitor the animal and in discussion with the NVC and NACWO we would humanely kill any animals exhibiting these signs to an abnormal extent or for an abnormal duration. For cardiovascular imaging (Protocol 4) we do not expect animals to suffer harm or exhibit suffering after these procedures, but if this is observed we will discuss with the NVS and NACWO, humanely kill the animal and review our methods and procedures to establish whether further refinements are required.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most (>90% of all animals used) of the animals will present with no more than mild severity or even subthreshold as these will be animals that are used to breed. However, a subset of animals that carry gene alterations that effect heart function may present with moderate severity at later stages of development or with age (1-5% of all animals used). All animals that undergo cardiac cryoinjury will experience a moderate severity procedure (<1% of all animals used). However, we will minimise any suffering by identifying early intervention timepoints to end the experiment, but still obtain the answers to the scientific questions posed.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Because we study the living organism and the interactions between many different cell types and the physical and

physiological conditions of blood flow, neuronal activation, etc, we are currently obliged to use animals and the zebrafish is the most appropriate and lowest neurophysiological organism which can be used.
Although there is currently no alternative to the studies proposed, we will continuously seek non-animal alternative options. We will do this by monitoring the scientific literature, and particularly materials generated by NC3Rs. Within the zebrafish community there is a strong culture of communication of advances that can be rapidly adopted, both via conventional methods (publications and conferences) and more novel methods (social media, particularly twitter).

Complementary to the animal studies described, we are developing collaborations with cell biologists to begin to develop cell-based studies to test some of our hypotheses (for example co-culturing astrocytes and endothelial cells to examine whether these develop kugeln). In time these data may reduce the need for some mechanistic in vivo studies.

However, the observations we make in the model can then be used to develop non-animal alternatives to test individual mechanisms. For example, it would not have been possible to identify the novel endothelial behaviour "kugeln" in any other organism, but we are now using cell-based assays coculturing endothelial cells with neurons and glial cells to attempt to develop in vitro assays to understand the mechanisms and reduce the number of animals used.

We are developing mathematical (in silico) models of neurovascular modelling (paper in preparation) which use the data we have collected to understand the mathematical relationships between neuronal activation, blood flow and vascular diameter. This would allow us to perform in silico experiments to reduce animal numbers used.
Which non-animal alternatives did you consider for use in this project?

We considered in vitro studies using human cultured cells, in silico models, but none of these are sufficiently yet advanced enough to model the complexity of the in vivo cardiovascular system.
Why were they not suitable?

We could not devise an in vitro system that reproduces the complex anatomic and physiological environment of the cardiovascular system. Specifically; there is no current non-animal model that contains endothelial, vascular mural cells, cardiac cells, inflammatory cells, neuronal cells and other cell types which also reproduces the physiology of the cardiovascular system (a beating heart with flowing blood and blood pressure, with the associated physical forces exerted on multiple tissues).

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

From previous licence animal usage based on the size of my research group and considering plans for future studies, and in discussion with NACWOs and acquarium staff. We have used the NC3Rs experimental design assistant (EDA) to perform group size calculations to establish the numbers of animals used for representative experiments, and based the likely number of experiments performed based on a forecast of 2-4 PhD students and postdoctoral scientists per year each performing a project involving 90% zebrafish experimentation.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I am using cutting edge microscopy that will gather more information from each animal than any previous studies, and time course studies where the same animal is studied over time, rather than using separate animals.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Our centre is a pioneer in the ability to rapidly genotype embryos to identify which to raise to adulthood, which has the ability to greatly reduce the numbers of adults maintained on this licence (as adult breeding stocks represent the majority of the regulated procedures). The protocols in this application include the ability to genotype animals earlier than 5dpf and so to discard animals before this stage if they are not required, which would greatly reduce the number of animals used according to the requirements of ASPA. We are refining our breeding including extending the age to which we maintain our animals which reduces the total number of animals maintained for breeding stocks. We are developing computer models of neurovascular coupling that may reduce the number of animal experiments required and we routinely share surplus embryos generated during matings with other groups to reduce both number of matings and numbers of embryos raised to adulthood.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The zebrafish is the animal model of lowest neurophysiological sensitivity that can be used for any studies of cardiovascular development, as lower animals than the zebrafish do not possess a cardiovascular system. This is why we use the zebrafish in preference to rodents or other mammalian species.

We use non-invasive imaging of transgenic animals to visualise the cardiovascular system (protocol 4). This reduces the harms associated with instrumentation for other methods of vascular imaging such as angiography. There is no current method to visualise the cardiovascular system in a way that is less harmful (the animals that undergo this do not display any sign of harm and are not subjected to a procedure that would be expected to induce suffering).

To study the response to cardiac injury (protocol 5), it is unfortunately necessary to induce cardiac damage by some method in some animal model. As above, the zebrafish is the lowest species in which this is possible, and the method (cryoinjury) has been established to reduce as much as possible the discomfort and suffering induced by the surgery, including the use of anaethesia and analgesia in recovery. This does not induce lasting harm; indeed unlike other animals, the cardiac injury repairs completely over weeks, leaving cardiac function normal after injury.

We will continue to seek to minimise harms by; expert husbandry and handling of our animals and by close monitoring of animals that have undergone any intervention.

Why can't you use animals that are less sentient?

There are no lower organisms that possess a functional cardiovascular system. Wherever possible we use embryos in place of older animals as these are likely to be less sentient.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will seek to refine our studies by discussing any new experiments with the NACWO and NVS to identify the optimal monitoring and post-op care and analgesia requirements, and to further improve these based on our experience and any relevant publications or other source of information.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will monitor the NC3Rs websites and publications and zebrafish husbandry publications and scientific conferences to identify new ways to refine our studies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

We will stay informed with the literature and developments in the field by monitoring NC3Rs literature and outputs and attending conferences in this area and in liaison with the NC3Rs Regional Programme Manager

Home Office

NON-TECHNICAL SUMMARY

229. Zebrafish models of retina development and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Zebrafish, Vision, Glial cells, Retina, Gliosis

Animal types	Life stages
Zebra fish	embryo, adult, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

In this project I aim to determine the role of specific cell adhesion molecules in the development and healthy function of the retina.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The central nervous system (CNS) consists of the brain, spinal cord and retina (eye). It controls most functions of the body and mind. Despite the importance of these tissues are made up of only two major cell types: neurons and glia. Most researchers focus on neurons because they are the electrical wires passing signals to perform daily functions. However, glial cells outnumber neurons in the CNS and they support neurons to make sure they are healthy and function properly. To make up the CNS, neurons and glia need to meet during development and make specific partnerships that last a lifetime. Glial cells have special shapes so that they can connect to the neurons. Changes in glial shape can make neurons sick and potentially lead to disease. I want to explore this really important question: how glial cells get their shape in the first place so we can make sure they keep it and support the neurons throughout life.

What outputs do you think you will see at the end of this project?

The work in this proposal will be of great relevance and interest to academics studying two of the fundamental questions in neurobiology: How is the nervous system built and what causes it to break down in disease? The data will provide for the first time a characterisation of glial morphology and their contact with specific synapses throughout development in real time in vivo. This work will provide significant and novel insights into the mechanisms that form the brain in vertebrates, and in many cases will provide information about the functions of genes known to, or suspected to, cause human neurological diseases and blindness.
REDACTED

Who or what will benefit from these outputs, and how?

This work will provide the following benefits;

A greater scientific understanding of the basic mechanisms of retina development
A better understanding of the causes of some human retina diseases
A greater awareness of zebrafish models of retina development and disease
These benefits are worthwhile because they will add to the sum of scientific knowledge in a fundamental and clinically important area, they will contribute to reducing, refining, or replacing animal studies in other species, and they may provide scientific insights that could lead to clinical improvements or new therapies in the future. These may be broken down as follows:
Short term benefits (1-2 years): Generation of novel zebrafish models which will provide tools with which to investigate retina development in zebrafish. Furthermore, these tools will be of wider interest to the developmental biology community (genes important in eye development often play roles in other developmental processes, and therefore these models can be shared within the scientific community)
Medium term benefits (3-5years): Analysis of retina development in the embryonic models generated will further our understanding of how the nervous system development is regulated. This will also provide the basis for future studies in zebrafish and/or other organisms refining our understanding of this regulation. Analysis of the potential roles for specific mutations in juvenile/adult zebrafish may also help us link mild developmental defects with abnormalities in visual function later in life.
Long term benefits (5+ years): Better defining the genetic regulation of retina development has several long-term implications. Together with the use of next-generation sequencing technologies identifying candidate causative mutations in blind patients, we can better understand why specific mutations may cause blindness, and how this can impact upon long-term vision health – with implications for genetic counselling and treatment plans. In addition, understanding how to build a retina in an embryo has important implications for regenerative studies, and

I expect that some candidate genes we identify as part of this project may represent good candidates to investigate as mediators of retina regeneration in zebrafish, with direct relevance to treatment of human degenerative diseases.

How will you look to maximise the outputs of this work?

-- All data generated from zebrafish will be published at the earliest reasonable opportunity. We have a strong track record for publishing their data in the highest impact journals REDACTED and will continue to strive for these standards of publication for all data arising from the use of zebrafish. The applicants are regularly asked to speak about their research at international scientific conferences, policy meetings, special interest groups and public events. We also regularly participate in outreach events to help promote the work we do and engage with key groups including patients, eye charities and research councils, politicians, and schools. These interactions have a significant bearing on our research objectives.
-- We are part of the establishment zebrafish community that is highly visible through their website. Any new data will be announced and displayed on the appropriate sections of the website describing zebrafish work. This includes unsuccessful approaches and "negative" data that may impact on the future experiments, and animal use, by others in the field.

Species and numbers of animals expected to be used

- Zebra fish: 24,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Zebrafish represent arguably the best model for carrying out the research described in the project plan. It has an excellent capacity for in vivo imaging of externally-fertilized embryos – since most of the phenotypic work in the proposal analyses retina development in embryos below the age of protection, this reduces the numbers of zebrafish used for much of the project to breeding stocks subject to potentially moderate visual testing procedures, but are not expected to generate significant discomfort. Furthermore, zebrafish also represents the organism with the lowest neurophysiological sensitivity possible for work that focuses on the nervous system.
All genetically manipulated animals which are to be taken over 120hpf, either for generation of transgenic line or analysis of phenotypes in larval and adult stages will be subject to regular intensive surveillance, and culled if they display behaviours or symptoms outlined in Protocols 2 and 3 (e.g. weight loss, arched back, cataracts) to prevent undue suffering. All other adults maintained for breeding stocks will be subject to the regular health checks as part of our normal animal maintenance procedures.

Typically, what will be done to an animal used in your project?

Most animals will be used for breeding and generating transgenic and/or mutant lines. Fish will be grown to selected time-points, where they may be culled for post-mortem analysis of fixed tissues or isolated cells from selected tissues.
Fish may also undergo non-invasive behavioural vision testing, where we film animals responding to visual stimuli, which will allow us to measure visual system function.

What are the expected impacts and/or adverse effects for the animals during your project?

Some of the mutant zebrafish lines may have dominantly or recessively inherited neurological phenotypes manifesting as a
progressive swimming deficit. In dominant models 50% will be affected, in recessive models 25% will be affected. In order to characterise neurological phenotypes in new zebrafish models it is important to fully document any abnormal behaviours. In order to characterise the progressive, transient or stable nature of the phenotype, a daily visual inspection of their behaviour will be performed and a record kept when appropriate, working closely with NACWO and veterinary staff. We anticipate there may be a moderate impairment of the wellbeing of the zebrafish disease models, however once their condition begins to deteriorate rapidly we will sacrifice them humanely via schedule one. If a severe abnormal behaviour
(e.g. inability to swim, severe listlessness) persists for longer than 10 minutes or severe emaciation is evident in an individual fish, this fish will be sacrificed immediately by a schedule one method. Any fish displaying a moderate phenotype or intermittent phenotype (including altered swimming activity or buoyancy, general listlessness), will be monitored closely and sacrificed by a schedule one method if the conditions deteriorates. The provoked response to feeding is a reliable way of determining the overall condition of a fish. Any phenotypic fish which do not respond to the introduction of food into the tank will be sacrificed by a schedule one method.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Most animals will be used for breeding and generating transgenic and/or mutant lines (Mild). To understand how the specific cell interaction we are studying is impaired with ageing requires animals to age, whether naturally, or in the presence of mutations that mimic premature ageing syndromes in humans. During the course of their life-span premature ageing will result in weight loss, arched back, cataracts (ageing phenotypes in later time points are considered moderate; non-recovery 0 %; mild 25 %; moderate 25%)
A small portion of animals will be imaged under anaesthesia post fin clipping or post skin incision (nonrecovery 0 %; mild 20 %; moderate 0%). Larvae may require embedding in agarose up to a maximum of 2 hours or up to 8hrs when the procedure is terminal (AC).

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Zebrafish represent an excellent model in which to study the eye, both in development and disease. The eye is structurally well-conserved between zebrafish and mammals, including the retina. In the zebrafish over 80% of genes with disease-causing mutations are conserved. The potential of the zebrafish model in clinical practice is extremely high, as evidenced by the successful identification of treatments for human disease using drug screening. The zebrafish is an eminent model for visualising cells in vivo as there are transgenic lines that label every neuron and glial cell, thereby facilitating the unambiguous identification of each cell type based on reporter expression. These transgenic lines facilitate the imaging of cell interactions in real time in vivo.
The proposed experiments need to be performed in vivo. The patterning of glial cells is a dynamic process requiring interactions in a heterogeneous environment, between neurons and glial cells, meaning it cannot be modelled in any meaningful sense in vitro.

Which non-animal alternatives did you consider for use in this project?

I have considered performing cell
culture studies to inform the interaction studies that will be performed in zebrafish. Where it is possible to identify candidate genes from literature searches of publications, I will do this to replace in vivo screens. In the instance studies have been done in other systems, and the data is available, these studies will not be repeated in my model.

Why were they not suitable?

It is
very difficult, if not impossible, to recreate the complexity of the in vivo state in this manner, and not possible to test genetic manipulations of glial patterning in this manner. In the instance studies have been done in other systems, and the data is available, these studies will not be repeated in my model. It should be noted I choose to use zebrafish embryos for this study, as a non-mammalian model organism, with the lowest neurophysiological sensitivity possible for work focusing on the nervous system.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals that we require for transgenesis and embryonic analysis are based on practical stock keeping considerations i.e. ensuring sufficient animals of the right sex and genotype for embryo collection and stock maintenance.
I have used data generated from previous PPLs (postdoc supervisor) to gain insight into the number of animals required per line and protocol. I established this using power calculations, based on http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html .

When the given protocol has not yet been tested by me, I will perform a small pilot study with maximum 3 animals per genotype as a refinement strategy. I have used data from the literature to estimate number of animals required for such new protocols. Overall, data suggest I need a maximum of 16 animals per genotype, per time point to allow statistical interpretation.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Only in selected cases will we perform phenotypic analysis on larvae or adult fish. We have sufficient pilot data from all studies to perform a priori power calculations to calculate group sizes. We will not initially exceed the predicted group sizes required to detect a 40% difference with 80% power (alpha of 0.05 as per convention). We will increase group sizes only if greater power is required, if the variance is greater than in pilot studies, or where the biological effect to be detected is less than 40%. Where we obtain data over time from the same animals, we will use statistics appropriate to repeated measures.
We will increase group sizes only if greater power is required, if the variance is greater than in pilot studies, or where the biological effect to be detected is less than 25%. Whenever possible we will use the same adult fish for in vivo assays, including non-invasive, non-harmful behaviour assays, followed by schedule 1 and in vitro cell culture assays, to try and reduce the number of fish that have to be grown in parallel for the different experiments. Furthermore, an advantage of working with zebrafish, both as larvae and adults is that we only need one animal to look at all tissues at the same time, on the same slide, which increases the quantity and relevance of the

information retrieved per animal. Where possible temporal studies will be performed using the same animal to gather serial data. As part of good laboratory practice, we will write an individual study plan (ISP) for each experiment involving regulated procedures.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The number of animals that we require for transgenesis and embryonic analysis are based on practical stock keeping considerations i.e. ensuring sufficient animals of the right sex and genotype for embryo collection and stock maintenance.

Many of the functional assays will be performed in vitro using isolated cells from different tissues of the same animal, allowing large amount of data to be collected from reduced number of animals To minimise the number of animals grown to an age protected by the Act, we will try and genotype the lines before the age of 5 days post-fertilization, after which they are protected by the Act, to identify unwanted genotypes to reduce the number of adults to grow. For example, embryos may be genotyped through micro-abrasion using the ZEG system.

Opportunities to reduce the number of animals required to develop a new line may be possible as new transgenic technologies are developed (e.g. genome editing). Breeding programmes to develop the new lines and intercrosses will be developed and we will use in-house databases (e.g. labtraks) to aid our efforts to maintain the minimal number of animals per line. Where necessary, gametes are expressed either to maintain fertility or to allow in vitro fertilisation, and numbers are determined by the requirements of maintaining fertility and preserving lines.

Furthermore, whenever possible we will use the same adult fish for in vivo assays, including non-invasive, non-harmful behaviour assays, followed by schedule 1 and in vitro cell culture assays, to try and reduce the number of fish that have to be grown in parallel for the different experiments.

Additionally, using such a small sized animal like the zebrafish allows us to look at all tissues at the same time, on the same slide, which increases the quantity and relevance of the information retrieved per animal. Where possible, temporal studies will be performed using the same animal to gather serial data.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The methods for generating mutants and transgenics are optimised to minimise numbers and adverse effects are minimal. Fin clipping and gamete expression methods rarely involve inducing any tissue damage or discomfort, but for all manipulative procedures animals are suitably anaesthetised (primarily for restraint) by addition of immersion anaesthetic to the medium (no instrumentation is required even for the anaesthetic) using an anaesthetic protocol suitable for the procedure and following best practise (Tricaine immersion). Where necessary euthanasia is by anaesthetic overdose.

Fin clipping for genotyping purposes will be performed whenever possible in larvae before independent feeding (before 5.2dpf (non-protected).

Any new procedures will be tested first in few animals in pilot experiment (up to 3 of each experimental and control group), under NACWO and NVS guidance.

Why can’t you use animals that are less sentient?

We will use the zebrafish in our research project because the proposed experiments are best performed in vivo. The patterning of glial cells is a dynamic process requiring interactions in a heterogeneous environment, between neurons and glial cells, meaning it cannot be modelled in any meaningful sense in vitro. I have considered performing cell culture studies to inform the interaction studies that will be performed in zebrafish. However, it is very difficult, if not impossible, to recreate the complexity of the in vivo state in this manner, and not possible to test genetic manipulations of glial patterning in this manner. It should be noted I choose to use zebrafish embryos for this study, as a non-mammalian model organism, with the lowest neurophysiological sensitivity possible for work focusing on the nervous system.
Studying vertebrate retinal development in this project can only be carried out using zebrafish as they have a retina similar in composition and organisation to humans, unlike invertebrate species. Further, this project relies on visualising dynamic interactions between different retinal cell types in real time in vivo. These experiments require fluorescent transgenes to specifically label these cell types which do not exist in other vertebrate species, like reptiles or amphibians. Furthermore, visualising these dynamic intercations rely on the optical clarity of the zebrafish embryo (before 120hpf), which is when retinal organisation takes place and is completed. As such, zebrafish are necessary to investigate retinal cell dynamics in development and achieve my specific scientific objectives.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I will follow the ARRIVE guidelines and use the checklist when reporting animal use in papers, therefore maximising information published and minimising unnecessary studies.
The methods for generating mutants and transgenics are optimised to minimise numbers and adverse effects are minimal. Zebrafish have a number of additional advantages for these studies, including their near transparency, genetic tractability, extensive genomic resources and small size. My previous work has provided experimental data to develop defined end-points for my protocols that will be employed in this PPL. In particular, my previous work has allowed me to optimise histology, so I know that the culling methods chosen are ideal for tissue preservation, required to address the scientific questions in this project. Scoring sheets and imaging (photographs) will be used to further refine the end-points. Finclipping for genotyping purposes will be performed whenever possible in larvae before independent feeding. Procedures will be tested first in few animals in pilot experiment (up to 3 of each experimental and control group).

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will follow the latest relevant literature and in house expertise as well as 3Rs resources and guidelines for zebrafish (https://norecopa.no/media/7384/zebrafish.pdf)

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

At my establishment, the excellent team of NACWOs, the director of the animal facility maintain regular contact with all PPL and PIL holders regarding latest advances and help us implement these. I will have regular discussions with them about my experiments and the 3Rs. I will visit the N3CRs website (https://www.nc3rs.org.uk/the-3rs) and continue to receive their newsletter to make myself aware of relevant advances.

Home Office

NON-TECHNICAL SUMMARY

230. Zebrafish: an alternative model for drug safety & efficacy

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Drug toxicity, Central nervous system disorders, Organ regeneration, Heart and kidney failure, Anaesthesia

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim is to use the zebrafish as an alternative, non-mammalian, model for assessing the safety and efficacy of new human drugs and human drug targets with a view to understanding how they might act in humans. This is achieved by studying the effects of drugs and drug target modification on the development, structure and function of major organs systems of the body, including nerves, heart, skeleton, kidneys, liver and digestive systems.

A retrospective assessment of these aims will be due by 13 April 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the
- project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A very high proportion of new human drugs fail to reach patients as they show some toxicity or are relatively ineffective. Moreover, many of these failures occur after a considerable amount of time, money and animals (usually mammals) have already been used to test their safety and effectiveness. Consequently better approaches for measuring drug toxicity and effectiveness are needed, which can be deployed early in drug development so that more effective and safer medicines reach the market, without wasting these valuable resources on drugs that subsequently fail. This is where the zebrafish, in particular the embryonic and larval forms (<14 days old) is proving invaluable for several important reasons. These reasons include: good genetic and physiological similarity to humans; transparent development allowing visualisation of organ function (e.g. heart rate and blood flow); ease of genetic manipulation allowing us to test the effects of altering gene function; and a small size (a few mm's) meaning we only need to use very small amounts of drugs for testing. This project aims to utilise these attributes to develop and apply approaches in which we can measure the toxicity and effectiveness of new drugs, and test the effects of potential drug target modification on major organs system of the body.

What outputs do you think you will see at the end of this project?

Overall, the outputs from this project will include: new information regarding drug safety, efficacy and target validation; new fundamental knowledge regarding the endpoints under investigation; new methodology to complement or in some cases replace the use of higher vertebrates for testing drug safety and efficacy; new data on the translational power of the zebrafish as an alternative model to mammals for these research areas; and publications, presentations and information for educational purposes.
Objective 1) Outputs will primarily be data on the safety profile of new candidate drugs with respect to seizure liability, developmental toxicology, cardiovascular, kidney toxicity and hearing and visual impairment. These data will be used for internal decision making processes in order to drop compounds with unacceptable liabilities, allow alterations in compound chemistry to be undertaken to avoid such liabilities, and to prioritise the further development of compounds showing a good side-effect profile. In the absence of such data, the first stage of *in vivo* testing (in which the integrated whole organismal biological activity of new drugs can be adequately assessed) would be in traditional mammalian toxicological and safety pharmacological assessments, and the selection of compounds for testing in such systems would be made in the absence of any other *in vivo* data. Consequently, the data provided in this project will enable better decision making regarding the progression of compounds into higher vertebrate testing programmes and reduce unnecessary testing in higher vertebrates on compounds destined to fail later in development
Objective 2) Outputs will primarily be the provision of new *in vivo* methods for the assessment of gastrointestinal toxicity, liver toxicity and neurobehavioral toxicity that may be deployed in the early stages of drug development provided sufficient levels of translation to higher vertebrates including man are achieved. Subsequently these methods may be used in the same way as the established assays in Objective 1 leading to gains in the efficiency and effectiveness of the drug discovery and development process. In all cases, assays are developed and validated for only high impact and/or high incidence adverse drug reactions and only in the absence of an appropriate existing approach to address a specific safety liability in a whole animal context early in the drug

development process. This means in all cases that at present the first stage of *in vivo* testing is undertaken in mammals at a relatively late stage of development after a considerable amount of resources have already been invested (animals, money and time). Using kidney toxicity as an example, the ultimate impact on organ function constitutes the extremely complex culmination of the influences of many different organs systems and initiating pathways. Firstly, delivery of the drug to the kidney (or relevant indirect target), is clearly dependent on the Absorption Distribution Metabolism and Elimination of the drug in question (e.g. protein binding, partitioning, metabolism etc). Once at the target the drug may exert its toxic influence via many different mechanisms, for example: physical occlusion via altered local haemodynamics through vasoconstriction or microthrombosis; crystal nephropathy caused by tubular uric acid accumulation; cellular injury for example due to rhabdomyolysis, acid/alkylosis or localised oxidative stress; or altered tubular membrane conductances or impaired solute transporter activities at multiple sites along the kidney nephron(s). This complexity simply cannot be recreated *in vitro* and as the most important consequence is an adverse impact on renal function, the development of *in vivo* measures of functionality are crucial.

Objective 3) Outputs will primarily be data on the efficacy profile of new candidate drugs with respect to the treatment of epilepsy, diabetes, kidney and hearing regeneration, alongside other general research and educational outputs. In common with Objective 1, data will be primarily used for internal decision making processes in order to drop compounds showing insufficient efficacy against the disease phenotype in question, compared with other compounds within that specific discovery project. Again, in common with assessment based upon safety liability, in the absence of zebrafish data the first stage of *in vivo* testing would be in a mammalian non–clinical efficacy model, for example a mammalian genetically-modified mouse model that recapitulates the disease phenotype of interest. Consequently, the selection of compounds for this *in vivo* efficacy assessment would be made in the absence of any prior *in vivo* data and as such the inclusion of zebrafish efficacy data allows better decision making regarding the progression of compounds and reduces unnecessary testing in higher vertebrates on compounds destined to fail later in development. For the specific disease models being used here, there are no appropriate *in vivo* approaches suitable for early stage efficacy assessment available. Using epilepsy as an example, as epilepsy is a complex multifactorial neurological disorder, *in vivo* models have been crucial in gaining a better understanding of ictal and inter-ictal processes, as well as for testing the effectiveness of antiepileptic drugs (AEDs). Traditionally the assessment of AED efficacy has focused on the use of pharmacologically or electrically-induced rodent seizure models, or rodent genetic models of specific epileptic syndromes such as Dravet syndrome (caused by a mutation in a voltage-gated Na^{2+} channel). The zebrafish has emerged over recent years as a viable lower vertebrate model for assessing AEC efficacy and unlike rodent models can be deployed early in the drug development process when compound numbers are high, and compound availability is low. In addition to its value as an efficacy assessment model, our use of video tracking and our development of the functional imaging approach promise the potential for identifying novel mechanisms of antiepileptic action by providing mechanistic data following specific pharmacological intervention. For example, using functional imaging we have already started to identify specific neural circuits that are more frequently activated during pro-convulsive compound exposure (e.g. the cerebellum and associated circuitry) and other circuits that appear particularly activated during exposure to specific pharmacological classes of compound (such as monoaminergic and cholinergic agents).

Consequently, the data outputs from such work are helping us to understand which circuits could be novel targets for AEDs, what drug-induced seizures look like (thus improving their detection during drug development), and what the neural-network processes (such as altered functional connectivity between certain brain regions or the recruitment of specific circuits associated with specific neurotransmission systems) leading from the resting state to the ictogenic state might be. Importantly regarding these 3 points: most AEDs target a limited number of ictogenic mechanisms, and as such there is still a massive unmet need for AEDs to treat refractory epilepsy; seizure liability still presents a major cause of CNSrelated attrition during drug development; and finally the exact mechanisms that lead to ictogenesis, or the transition from local excitation to widespread hyper-synchronicity typical of a seizure are still poorly understood. Collectively, therefore, our work will further the fundamental understanding of the development, progression and treatment of seizures and the chronic seizurogenic syndrome epilepsy in humans.

Objective 4) The main output from this objective will be the provision of *in vivo* data on the link between genotype and phenotype in zebrafish in relation to high impact and/or incidence human diseases. Generation of these data will serve three main purposes: firstly they will allow drug discovery scientists to make data-driven decisions about which genetic targets to prioritise for further evaluation in genetically modified mouse models (currently decisions are made using only *in silico* and i*n vitro* data meaning only a limited number of targets can be pursued *in vivo*, and some targets subsequently fail due to poor *in vitro* to *in vivo* translation); secondly, in some cases they could

provide zebrafish-based disease models that can be used as alternatives to mouse-based disease models and allow assessment of multiple novel drugs at a much earlier stage of discovery; and thirdly, these data will help to improve our understanding of the genetic basis of the targeted human disease for example by allowing exploration of the link between a gene mutation identified from clinical assessments of patients, and an *in vivo* phenotype that has not yet been described in a non-clinical model. Demonstration of a similarity between the clinical and non-clinical phenotypes supports the involvement of mutation of this gene in the disease in question. Specifically, no CRISPR-based knockout and subsequent systematic organ development and functional data in zebrafish exists for any of the genes being proposed here. For those genes identified in patients suffering from heart or renal failure, for example, there are no published studies in which gene knockout followed by detailed phenotypic analysis and measurement of cardiovascular (e.g. heart rate, blood flow, vasodilation/constriction) or renal function/pathology (e.g. glomerular filtration rate, podocyte number) are available. Consequently to date there are no data to support a functional link other than that from *in silico* or *in vitro* assessments, and therefore limited data to support their further development in mammalian models as potential targets for therapeutic intervention, or to support further research into the molecular mechanisms involved in the transition from gene mutation to altered protein function. This project will provide these data. For those genes identified as associated with ADHD and ASD, there is still a lack of fundamental data on which brain structures and circuits, or which neurotransmitter systems underpin these multifactorial diseases, and how alterations in these results in the human disease phenotype. Using our functional imaging approach we will generate data on altered brain circuitry associated with mutation of several risk genes compared with non-mutated animals, and assess the resultant effect on dopaminergic signalling in the vertebrate brain. This will provide an insight into the factors linking genotype with phenotype in these conditions, providing further knowledge of the aetiology of these diseases as well as potential targets for further therapeutic intervention.
Objective 5) The work undertaken under this objective will provide researchers with the first definitive data on appropriate anaesthetic and analgesic agents and treatment regimes in embryo-larval zebrafish (the most widely used species/life stage of laboratory fish), improving understanding of appropriate anaesthetic doses and induction/recovery times, as well as providing information on post-surgical analgesia. The data generated will also help to refine the accuracy of visual indicators of anaesthetic status for in-procedure monitoring, which are routinely used during such procedures. These data will be generated in zebrafish, but will provide a foundation for refining anaesthetic protocols in other species of fish. We will provide the first detailed data on the effect of agents on specific zebrafish neural circuits allowing CNS researchers to select the most appropriate agents for their research. These data will also provide fundamental information on fish (un)consciousness and pain perception, and in addition, the functional imaging component will provide fish CNS researchers with a potential non-invasive replacement for implanted electrode techniques in CNS functional studies.

Who or what will benefit from these outputs, and how?

Industry will benefit from the provision of techniques, data and improved knowledge of their drugs to enable faster and more effective decisions to be made about the potential of certain projects. This is turn will lead to economic and animal savings destined to be used for studies on drugs that subsequently fail. The wider public will benefit through the generation of safer more efficacious medicines, more efficiently and rapidly than would otherwise be possible using traditional *in vivo* testing approaches.
The scientific community will be benefit from improved knowledge, development of techniques and access to publications and presentations of our research findings.
Objective 1) These data will allow safety assessment scientists within our collaborator laboratories to make decisions regarding the further development of certain compounds based on their safety profile. The data generated will allow them to drop compounds, redesign chemistry or prioritise additional safety assessment tests this leading to increased efficiency (including saving animals, time and money used on compounds with safety liabilities) and success in the development of new medicines for patient use. These gains are expected to be immediate. These gains are expected to be within the life span of the current project, as has been the case with previous licences.
Objective 2) It is anticipated that these outputs will be used by collaborator and other laboratories both for drug screening purposes, but also for other purposes as has been the case in the past for example with the use of cardiovascular screening approaches in the assessment of the effects of environmental chemical exposure in fish.
Objective 3) These data will be used by discovery biologists within our collaborator laboratories to make decisions

regarding the further development of certain compounds based on their efficacy profile. This data will allow them to assess many more compounds than is currently possible using traditional i*n vivo* model systems with the aim of selecting the most efficacious for further testing and development, for example in mouse disease models. These gains are expected to be immediate in the case of current models, and within the time scale of the current project in the case of models that require additional development time.
Objective 4) Discovery scientists within our collaborating laboratories will use these data to contribute towards the discovery of new drug targets for high impact human diseases. This information is likely to be used alongside *in silico* and *in vitro* data on new drug targets to justify the generation of mouse models of the most promising gene targets thus allowing more effective evidence-based triaging. In addition, in some cases new screening models may be produced in which new candidate drugs can be tested at a higher throughout than is currently possible with mouse models used in target validation and early drug testing. Data generated from this objective are likely to be the most commercially sensitive, however wherever possible general research and educational outputs will be provided to increase learning about the function of these genes and their roles in human disease aetiology. These gains are expected to be within the life span of the current project, as has been the case with previous licences.
Objective 5) Researchers using fish models will gain the first definitive data on appropriate anaesthetic and analgesic agents and treatment regimes in embryo-larval zebrafish (the most widely used species/lifestage) and zebrafish neuroscientists with data to assess compatibility with their work as well as fundamental information on fish (un)consciousness and pain perception. Although focused on zebrafish, it is anticipated that this project will also provide valuable translational data for aiding decision making in other fish species for example by helping to refine the accuracy of visual indicators of anaesthetic status for in-procedure monitoring by veterinary surgeons, or by providing a starting point for large-scale fish anaesthesia for transportation, vaccination, tagging etc. in the ornamental fish industry.

How will you look to maximise the outputs of this work?

We are a highly collaborative group and always seek to exploit opportunities for collaborative working.
This is illustrated by a number of publications in which some of our in house developed techniques have been used for studies outside of our core aims of assessing drug safety and efficacy (e.g. for assessing the physiological impact of environmental contaminants).
In addition, we are active presenters at scientific meetings and other universities, for example delivering presentations at international conferences, specialist meetings, local seminars and outreach events.
Despite a close working relationship with pharmaceutical companies, as a group it is our policy to publish (as evidenced by our collectively strong publication records, given our industry focus), wherever possible in peer-reviewed journals. This approach maximises the benefit of developments made under this project licence to the scientific community, and contributes towards the body of evidence supporting the usefulness (and ultimately regulatory acceptance) of the zebrafish as a mammalian alternative for drug safety and efficacy testing.

Species and numbers of animals expected to be used

- Zebra fish: 104500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The zebrafish has emerged as a credible vertebrate model for the assessment of human drug safety and efficacy. In particular, the embryo-larval possesses a number of important attributes that infer advantages over more traditional, higher vertebrate (e.g. mammalian) models. These include: rapid and transparent development meaning biological processes can be easily be seen under a microscope; good genetic similarity with humans (a reported 83% of human disease-related genes are found in the zebrafish); and a small size meaning they do not require a large amount of space or large amounts of drug for use in tests.

Typically, what will be done to an animal used in your project?

Procedures used are: modifying a gene and assessing the effect this has on an animal or its organs; exposing zebrafish to drugs by immersion or injection and assessing the effect this has on an animal or its organs; assessing whether drugs are effective in treating fish in which the development or function of an organ has been compromised.
Apart from the provision of adult broodstock, in the vast majority of cases, these procedures will be undertaken in young zebrafish embryos and larvae (typically <10 days old and a few mm's in length). In many cases, the duration of the experiments is short (e.g. 1 hour-2 days) with the longest part of all procedures associated with exposure to the drugs to ensure sufficient absorption into the animal.

What are the expected impacts and/or adverse effects for the animals during your project?

In many cases, the effects of genetic modification, drug exposure or specific procedures will be mild with little more than transient effects on the welfare of the animals used. For example, over the past 4 years 46% of the animals used have been classified as of mild severity. The following adverse effects are based upon the worst case scenario where a new drug is being tested in larval zebrafish (<14dpf) without prior knowledge of its toxicity or a new disease model has been created:
Death
Morphological defects (estimated duration in protected animals 8 hours)
Seizures (<2 hours)
Moderate water retention (lifespan of the animal)
Abnormal posture or loss of balance (estimated duration 2 days)
Any of these observed in adult animals would lead to those animals begin humanely killed immediately.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severities range from mild through to severe across all protocols. The numbers expected within each category can be estimated from previous figures (2016-2019) as an average across all protocols:
Mild ~45%
Moderate 42%
Severe ~11%
Non-recovery ~2%
All animals used are zebrafish and in the vast majority of cases will be embryos and larvae of <14 days old. Specifically, >99% of exposures will be undertaken in animals of <14dpf, with adult animals only used under specific circumstances (e.g. for the assessment of later-stage endpoints for developmental toxicity), or for the production of brood stock to supply embryos and larvae for subsequent drug testing.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 13 April 2026

The PPL holder will be required to disclose:
- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Drug safety assessment and a true representation of human disease characteristics require intact animal models to accurately recreate a full organism response. For example, seizures and epilepsy are complex neurological processes and, as such, *in vivo* models with intact nervous system structure and function are crucial for furthering our understanding and for confidently detecting seizures as a side effect of new drugs.

Similarly the overall aim of the target validation work is to use the zebrafish as the first *in vivo* model for assessing the likelihood of new drug target working. Cell-based approaches are suitable for understanding basic function, but the complexity of a whole animal is vital to fully understand the structural and functional effects of gene alteration and interactions with new drugs. Normally mice are used for this but this project aims to use the zebrafish as a precursor in order to ensure only the most scientifically-appropriate and relevant mouse models are then generated for further study.

Which non-animal alternatives did you consider for use in this project?

The ultimate purpose of this project is to understand the effects of drugs and the biology of drug targets in humans. With increasing knowledge through the project, there is the potential for replacement of protected with non-protected animals. A good example is with our recent advances in brain imaging in which we may be able to detect and measure seizures at 4dpf instead of 7dpf. Similarly, in the target validation work our strategy is to use non-protected life-stages of zebrafish initially, and only use protected life stages when and where is absolutely necessary in order to answer the specific biological question being posed. This approach makes sense as the specific properties of the embryo-larval zebrafish allow target validation to be undertaken more simply and rapidly than in older animals, or in mice.

Why were they not suitable?

The endpoints selected represent those for which no appropriate alternatives exist to bridge the gap between cell based and traditional (e.g. mammalian) animal testing models. For example, the emphasis in the drug safety assays is on functional assessment of side effects, and thus these types of assessments in their entirety cannot be recreated using cell based assays. Using seizures and epilepsy as an example, although some invertebrate models have been proposed (e.g. fruit flies) these lack the level of nervous system complexity and human translatability that the zebrafish offers and the larval
zebrafish is currently the most refined animal in which the detection of, and mechanistic studies into, seizures can be undertaken. In this respect, the overall aim of this project is reduce or replace the use of rats and mice for this purpose. Our approaches, therefore, offer the best level of refinement for this process and this is supported by existing published and recently derived data.

Similarly with the target validation work, although some invertebrate models have been proposed, these broadly lack the level of physiological complexity and human translatability that the zebrafish offers, and again the zebrafish offers the most refined vertebrate species for use in this context. In common with the drug safety and effectiveness tests, the aim of the target validation work is to use the zebrafish as a lower vertebrate bridge from cell based to whole animal translation to ensure evidence for the link between gene and target is strong prior to investing in mouse models.

A retrospective assessment of replacement will be due by 13 April 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers estimated reflect our average annual returns over the last 4 years with a 30% increase added in anticipation of an expected increase in demand, especially with respect to the target validation work which is in its relative infancy in our group.
In mitigation, our data reflect the fact that embryo-larval zebrafish have higher throughput amenability, that we use individual animals as the experimental unit in our work rather than using the tank as the experimental replicate measure, and that we use the most appropriate age of animal to meet the requirements of the endpoint under investigation. For example, method development work and model validation exercises suggest that 7 days is the earliest age for assessing particular behaviours, although our proposed work to validate the use of brain imaging for seizure detection may mean this is reduced to 4dpf.
Adult fish are only used in drug exposures under rare circumstances (e.g. <1% of animals used), for example where the investigation of a specific endpoint requires analysis of an older animal (e.g. for developmental toxicity assessment).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Despite the relatively high numbers proposed, for every endpoint investigated the numbers used will be minimised by employing the following control measures:
Careful experimental design (e.g. utilising shared control groups and/or intra-individual controls where possible, power calculations, and previously generated datasets) has allowed the development of methods to obtain usable data with the minimum number of animals.
To reduce embryo-larval numbers used in established assays (seizure liability and developmental toxicology), the assessment of general toxicity (via the Maximum Tolerated Concentration or MTC assessment) is integrated into the main assessments rather than being conducted as a separate study. For any adult fish exposures, and during larval fish assay development, however, a pilot study is undertaken using a reduced number of animals to establish an appropriate exposure regimen, prior to commencing the main study. For non-proprietary drugs, this is supplemented with information on toxicity from the published literature. The overall aim is to minimise inappropriate drug-exposure related adverse effects in the definitive assessments.
Where appropriate, power calculations will be used, although when assessing drugs with unknown toxicological and pharmacological properties, the magnitude of response is less predictable. In such cases, experimental experience will be used to judge an appropriate number of animals to use in the first instance, after which standard operating procedures will be used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

A key strategy is to maximise the amount of information extracted from each animal used. Examples include using the same animals for morphological assessment and histopathology in the target validation work, use of toxicity data for multiple assays employing the same test compound, and the measurement of multiple endpoints in the same animals. The latter approach is important; as well as providing more scientifically-relevant and robust data, this also serves to increase the throughput of any assay developed which is central to the success of any work that is undertaken using the zebrafish as an alternative model.
Ultimately, the overall purpose is to reduce the number of tests undertaken unnecessarily on mammals with drugs that will eventually fail due to a poor side effect profile or low efficacy, or for which the evidence for target translation to humans is poor.
A retrospective assessment of reduction will be due by 13 April 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

All of the work contained within this project uses the zebrafish, and in the vast majority of cases, embryo-larval fish will be used in exposures (>99% expected to be <14dpf). Only under very specific circumstances will older animals be used, for example for the investigation of a specific endpoint requiring analysis of an older animal (e.g. for developmental toxicity), or when genetically-modified fish are maintained and bred to provide embryos and larvae for subsequent drug testing. With regards to the specific models and techniques to be used:
Basic brain structure and function of relevance to seizures and epilepsy are highly conserved between mammals and zebrafish. The actual measurement of seizures in zebrafish are non-invasive (behaviour and imaging) in contrast with the use of implanted electrode recording in the brains of rodents.
The Zebrafish developmental toxicity assay has shown high predictive value against rats and rabbits traditionally used for developmental toxicity testing. Assessing development defects in zebrafish is noninvasive (imaging) and can be undertaken without the need to sacrifice pregnant rats or rabbits.
Zebrafish embryo-larvae possess an anatomically simple kidney that is easily accessed and functionally comparable to mammals. The actual measurement of renal function in zebrafish is noninvasive (imaging), and due to *ex vivo* development can be observed without the need for invasive surgery.
Due to external and transparent development, cardiovascular endpoints (e.g. heart beats, blood flow and vessel diameter) can be observed without the need for invasive procedures as is the case in mammalian models.
The lateral line hair cells of the zebrafish are structurally and functionally comparable with those of the inner ear of mammals, and have been shown to be sensitive to the action of ototoxic drugs. These can also be observed without termination of the animal and sectioning of the head, as is required in mammals.
The zebrafish visual system shows good comparability with humans having similar retinal structure and an abundance of cones providing rich colour vision. The measurement of visual acuity in zebrafish is non-invasive (imaging), and can be measured using passive assessment of behavioural responsiveness without the need for restrictive experimental chamber containment, for example in the case of rat based assays.
The zebrafish gastrointestinal system shows anatomical and cellular architecture that is similar to that of the human tract. Crucially the transparent nature of the body wall in larvae allows non-invasive visualisation of contractions following drug treatment.
The zebrafish liver is functionally comparable with that of the mammalian liver, even in larval life stages. In common with other endpoints, observation of liver structure and function can be undertaken noninvasively in embryo-larvae due to their optical transparency.
The zebrafish pancreas is structurally and functionally comparable with mammals and can be observed non-invasively through the use of imaging in transgenic zebrafish models in which specific cell types are fluorescently-labelled.
In addition to the study of seizures and epilepsy, the zebrafish is emerging as a valuable model for other neurological and behavioural disorders, for example into the genetic basis of autism spectrum disorder (ASD) and attention defect hyperactivity disorder (ADHD). The use of non-invasive brain imaging can provide better insights into potential therapeutic targets.

Why can’t you use animals that are less sentient?

Fish are arguably the least complex vertebrates that are generally regarded as an appropriate surrogate species for the assessment of mammalian (including human) drug safety and efficacy. More specifically, the zebrafish, particularly in the embryonic and larval stages, possess a number of features that make them particularly valuable

for use in assays that address drug safety and efficacy. In particular they possess functionally comparable major organ systems; they exhibit good genetic comparability with mammals in key regions; they are easily genetically manipulated; and they are transparent and small meaning organ function can be easily visualised, and small amounts of compound can be used to undertake small-scale studies which are amenable for use early in the development process of any given drug.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

In addition to the use of generic indicators of adverse effects as humane endpoints, information is gathered to provide more specific humane endpoints for use on both existing and future models.
Protocol 5 is rated as severe as many of the new candidate drugs begin tested have very few if any available information on their toxicology. Consequently, there is the possibility of severe adverse effects including death the first time these drugs are tested. For this protocol only immature life stages (no adults) will be used. As the larval zebrafish is the first 'whole animal' in which any testing will be undertaken it is difficult to predict the likelihood of adverse effects in advance of testing. Where prior data are available (rare), the concentration range used initially will be refined accordingly. Where not available, standard concentration ranges are used for each assay which are further refined based on compound solubility. In addition, exposure periods are either very short and the animals are under almost constant observation with the animal begin humanely killed as soon as the aims of the experiment are achieved (e.g. to assess seizure liability), exposure is commenced on day 0 (before) protection and the animals are terminated on day 5 when aims of the experiment are achieved (developmental toxicity), or for longer exposure in protected animals, frequent checks for adverse effects and limits of severity (e.g. hourly for the first 6 hours of exposure), extending to a standard regime of at least 2 times in any 24 hour period with no more than 14 hours between any two checks are undertaken. In all cases the Maximum Tolerated Concentration (MTC) of the drug will be ascertained allowing adjustment of the dose regimen in subsequent experiments.
For genetic modification, animals will be sacrificed as soon as the experimental aims are reached. in many cases, these are fluorescent reporter lines showing no adverse effects of genetic modification. For target validation, phenotypes that are considered sufficiently mild to grow on for further assessment will only be grown on when the specific requirements of the experiment necessitate it. For example: to assess for a phenotype that emerges later in development; to allow phenotypic analysis in adults without the influence of active development or to provide sufficient tissue for detailed histological analysis; to provide brood stock for drug efficacy screening; or to assess trans-generational effects. It is estimated that this will apply to less than 10% of the genes investigated.
The use of fin clipping is widespread for the purposes of genotyping fish, but a growing number of publications are proposing skin swabbing as a more refined method, especially in older animals. Despite this, there is still some debate around which method is less stressful for the fish and if skin swabbing is appropriate in earlier life stages. As part of this project we will further investigate the appropriateness of skin swabbing for the purpose of genotyping individual zebrafish, particularity at younger life stages (as well as a very new method involving skin sampling from larvae via non-contact collection of sloughed cells). Provided adequate comparability in terms of DNA quality, contamination and sample size between skin swabbing and fin clipping are achieved, we will move to skin swabbing for all genotyping work under this licence. These data will also inform existing projects within our facility that still routinely use fin clipping for the purposes of genotyping zebrafish.
The use of individual animals as our experimental replicate often necessitates the housing of animals in individual wells of micro-plates during drug exposure. Prior to commencing the experiment, embryolarval stocks are held in groups of around 50 animals in Petri-dishes (circa. 35 ml volume) until experimental use where they may be transferred to micro-plates. Typically 24 (maximum volume ~ 3 ml) or 48 well (maximum volume ~ 1.6 ml) plates are used for animals up to 8dpf, and larger volume wells used for older animals such as 12 well plates (maximum volume ~6 ml) or Petridishes up to 14 dpf.
With full validation, and assuming good concordance with clinical outcome, much of this work could lead to the a reduction or even the replacement of mammalian *in vivo* techniques, thus replacing animals of higher neurophysiological development with those of lower sentience, or replacement of protected with non-protected (pre free feeding) embryo-larval forms. Indeed progress made under previous licence(s) has meant that the use of established assays in drug safety screening programmes within industry allows, in some cases, deselection of drugs with poor safety profiles prior to testing in mammals, and in other cases prioritisation of endpoint-specific studies earlier in development than would otherwise be the case. This is expected to be expanded further with the addition of genetic target validation work into our experiential portfolio.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We adhere to the Home Office, UKRC and NC3Rs guidance on the application of the 3Rs to our research. We also adhere to the PREPARE guidelines for animal experimentation and ARRIVE guidelines for the publication of our *in vivo* experimental findings.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

As a laboratory we are highly active in the promotion and application of the 3Rs to our work as evidenced by some of our recent publications and presentations including at fora promoting the use of alternative models in toxicology and pharmacology.
Moreover, some of work we undertake is directly funded by the NC3Rs and we have been active in the development of refined techniques throughout the tenure of previous licences, such as the development of the non-invasive neural imaging approach in non-protected larvae.
We will continue this approach throughout the tenure of this licence by keeping up to date through publications, conference attendance, collaboration and funding body interactions.
A retrospective assessment of refinement will be due by 13 April 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Home Office

NON-TECHNICAL SUMMARY

231. Microvesicles and the prevention of tissue wasting

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Stem Cells, Cancer, Microvesicles, Ageing, Progeria

Animal types	Life stages
Mice	adult, embryo, juvenile, aged, neonate
Rats	juvenile, adult, aged, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The objective of the project is to explore the clinical benefit of stem cell secretory factors on age related degenerative diseases and those associated with cancer treatment. To facilitate the safe translation of pre-clinical testing to human clinical testing, animal testing is necessary in this project.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Previously studies have shown that molecules produced by stem cell when given to the person from whom the cells were first taken (autologous treatment) had therapeutic uses. However, most work has concentrated on degenerative illnesses including multiple sclerosis and injury conditions like joint damage.

This project will serve to explore properties of the molecules produced by stem cells and expand the range of conditions that it could help. Specifically, we will now focus on age related tissue loss and cancer treatment. This will provide pre-clinical data on safety and efficacy to facilitate early clinical application of therapy in diseases which currently have few treatment options.

The clinical use of molecules produced by stem cell provides an attractive, less invasive, safer alternative (minimising cancer, reducing immunological risks) to stem cell transplantation.

Our approach is novel since it is aimed at ultimately being used in a large number of people. To this end, we have been mindful that any approach that we investigate has to be amenable to almost industrial level scaling. Our approach outlined in this license is unique since both the source of regenerative molecules, i.e. the stem cells, and their particular properties support this primary requirement. We have been mindful that any stem cell that we use has to be capable of large scale culture in order for it to be used in a clinical setting. To that end, we will use stem cells which can be maintained for long periods of time before they are unable to divide. Additionally, a novel feature of our work, in the development of potential therapeutics, has been the understanding of stem cell activity, which depend on their environment. We have shown in a number of studies, that stressing stem cells, changes their secretory properties, which prime them to make molecules that show superior regenerative capacity than cells maintained in unstressed conditions.

What outputs do you think you will see at the end of this project?

This project will serve to explore stem cell conditioned media and expand the range of conditions that it could help. Specifically, we will now focus on age related tissue loss and cancer chemotherapy induced tissue loss. This will provide pre-clinical data on safety and efficacy to facilitate early clinical application of therapy in diseases which currently have few treatment options.

The key outputs will be the generation of new information, which will be communicated via publications in peer reviewed international journals.

Who or what will benefit from these outputs, and how?

The clinical use of differentiated or undifferentiated stem cell conditioned media provides an attractive, less invasive, safer alternative (minimising cancer, reducing immunological risks) to stem cell transplantation. The benefits could be in future be reaped by those who suffer muscle loss as a consequence chemotherapy use or during the ageing process.

However, this is only the first step towards use in the community. Future studies will require safety testing a process that is likely to take many years after the successful completion of this project.

How will you look to maximise the outputs of this work?

We will communicate the results arising from this study by presenting our progress at both national and international conferences and meetings as well as communicating completed studies in the form of publications.

Species and numbers of animals expected to be used

- Mice: 6700
- Rats: 1500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Rodents are being used in this project as they can be used to develop the symptoms of the diseases that are the focus of this project: muscle wasting associated with cancer chemotherapy-induced muscle wasting and muscle wasting associated with ageing.
For the cancer chemotherapy-induced muscle wasting work, we will focus on rodents at stages of life which mimic when humans could suffer from this condition which will be juvenile and adult stages.
For the work on ageing, we will focus on older animals but will also work on rodents that have genetic backgrounds that result in them ageing in an accelerated manner.

Typically, what will be done to an animal used in your project?

Typically, rodents will be bred so that they have the correct genetic background. They will then be induced to develop a disease state, this is be achieved either through breeding or exposure to a chemical.
Thereafter they will be treated with stem cells or molecules produced by stem cells which have potential therapeutic properties.
The treatment will be allowed to have time to work, during which time the rodents will be monitored to assess the impact of the treatment.
The experiment will be terminated at a set period, typically. The animals will then undergo physiological examination which will allow us to gauge how well the muscle are working. Thereafter the animals will be killed and tissues collected for further investigation aimed at revealing how the treatment has altered the course of the disease.

What are the expected impacts and/or adverse effects for the animals during your project?

It is likely that in all models we will induce loss of body weight which will be monitored during the course of the experiments. We plan to terminate the experiment when the intervention to induce tissue wasting results in greater than 20% of body weight loss compared to control mice.
Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The most severe category is moderate which will be experience by no more than 20% of the animals in this proposal.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The objective of the project is to explore the clinical benefit of stem cell secretory factors on age related degenerative diseases and cancer chemotherapy-induced muscle wasting induced tissue wasting. To facilitate the safe translation of pre-clinical testing to human clinical testing, animal testing is necessary in this project.

Which non-animal alternatives did you consider for use in this project?

With some aspects of the project, experimental animals can be replaced by in-vitro testing, and as outlined in the programme of work such in-vitro testing will replace intact animal experimentation wherever possible in the project. Such in-vitro testing will include appropriate dissociated cell culture, cell line testing and testing on organotypic slice culture preparations appropriate for each disease model.

Why were they not suitable?

While in-vitro studies are very informative on the impact of a disease or condition on a particular tissue, they have very little value when the nature of the disease or condition acts in the whole body.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For quantitative experiments sample size will be set statistical predictions based on the difference we want to detect to give us an indication that a treatment is working. We will draw on our work conducted using the same animal models and how the experiments were run to give similar but unique outcomes.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

More generally, advice will be sought from our Consultant Statistician, where appropriate, to ensure a statistical design that is used is efficient and minimises the number of animals required, yet maintains sufficient precision and power. For example, advice will be sought when a specific pre-clinical model introduces large subject-to-subject variation.
Furthermore, our studies have shown that the impact of ageing and cancer chemotherapy-induced wasting effect more than just the primary tissue under investigation, that being skeletal muscle. Therefore, in order for an intervention to be examined further for possible use in humans, it is now apparent that an intervention should be examine at the organismal level as well as a range of tissues.
We have developed a pipeline that extracts the maximal amount of data from each animal by not only determining its behaviour and movement prior to its death, but then to isolate as many tissues as possible. This approach means that a single animal will generate data related to a wide variety of bodily functions.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The proposal aims to minimize animal usage by drawing on our extensive experience in this area of research. These insights will be deployed not only to keep the number of mice to their lowest possible number but also to conduct the experiments in the shortest time span thus minimizing animal suffering.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The choice of models employed in the project also reflects the wide variety of degenerative diseases and components of damage they inflict. Thus the models selected provide the most data on whether stem cells or factors produced by stem cells can induce repair of tissue damage.

Why can't you use animals that are less sentient?

Small rodents (mouse: rat) are the simplest appropriate pre-clinical models to study these diseases and their potential amelioration. While there are species differences in physiology between humans and rodents, these are minimal and the use of species genetically closer to humans is not required by the appropriate regulatory bodies and thus is not proposed as part of this project. The mouse is the species of choice due to availability of appropriate genetically modified lines that phenotypically display progeria (accelerated ageing). Furthermore genetically modified mice, either through gene knock-out or gene over-expression studies allows us to develop mechanistic explanations at the molecular level of the processes of cachexia and ageing.
Non-mammalian models including nematode worms and fruitflies do not share the same physiological parameters as humans and are thus not informative for this type of study.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

During the tenure of this license other progeria models may become available and appropriate advice will be sought as to their suitability for this work and on any welfare issues pertaining to their phenotype.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will seek to carry out the experiments using best practice advised by differing parties including:
Laboratory Animal Science Association (LASA) for best practice guidelines for administration of substances and genetically modified mouse welfare guidelines.
We will also turn to the Workman guidelines for cancer research (https://www.nature.com/articles/6605642) as these principles are particularly relevant to our study as they describe welfare issues and how to use best practice for models which experience tissue wasting, the core focus of this proposal. Please see:
Workman P, Balmain A, Hickman JA, McNally NJ, Rohas AM, Mitchison NA, Pierrepoint CG, Raymond R, Rowlatt C, Stephens TC, Wallace J (1988) UKCCCR guidelines for the welfare of animals in experimental neoplasia. Br J Cancer 58: 109–113

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during

the project?

We will seek advice from the Home Office inspector as well the named veterinary surgeon for alternative approaches using either less sentiment animals or non-animal systems that allow us to achieve our goals.