Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted April – June 2023 that require a retrospective assessment

Contents

1. Infection and Immunity of Avian Viruses

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, ortheir effects, in man, animals or plants
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
  - Assessment, detection, regulation or modification of physiological conditions in man,animals or plants
  - 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 aimsmentioned in paragraph (b)
  - Protection of the natural environment in the interests of the health or welfare of man oranimals

Key words

Avian viral diseases, Zoonosis, Vaccines, Antivirals, Probiotics.

Animal types	Life stages
Domestic fowl (Gallus gallus domesticus)	embryo, juvenile, adult, neonate
Duck (Anas Platyrhynchos)	embryo, juvenile, 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.

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 aim of this project is to improve disease control systems against several important avian viruses by defining how avian influenza viruses (AIVs) cause disease and persist in poultry. Additionally, determining the effects of co-infection with AIVs and other avian

viruses on morbidity, mortality, and transmission, and by developing novel mitigation approaches (vaccines and antivirals) will help reduceproduction losses and zoonotic or pandemic threats from these avian viruses.

A retrospective assessment of these aims will be due by 18 October 2028

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 advancedor 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?

Poultry production is a critical sector for food security, economic development and poverty reduction,but it faces significant challenges due to avian viruses such as AIVs. These viruses are posing a significant threat to poultry, and their spread is mainly due to migratory wild birds that can transmit theviruses to domestic poultry flocks. The current epidemic of high pathogenicity avian influenza H5N1 virus has led to the death or culling of over 200 million domestic poultry worldwide during 2022/2023. To control the spread of AIVs, pre-emptive measures such as mass culling of infected and potential contact flocks are taken, which can result in significant economic losses. We aim to develop a comprehensive knowledge base of AIVs circulating in wild birds and poultry to develop more effectivedisease control systems, including highly effective vaccines that provide full protection from AIVs together with other major viral diseases affecting poultry production.

Co-infection with multiple avian viruses is another significant threat to poultry, exacerbating the severityof the disease and reducing vaccine efficacy. We aim to study the mechanisms of co-infection and howthey impact AIVs persistence in poultry.

Vaccines are essential tools for reducing the impact of viral diseases in poultry, but the administration of multiple doses for each disease can be costly and repeat vaccination programmes also stressful to animals. We aim to improve the effectiveness and multivalency of poultry vaccines, so one or two vaccines administered at the hatchery can provide long-lasting protection against AIVs along with otherimportant avian viral diseases. Additionally, we plan to explore the use of cost-effective antiviral and probiotic strategies to minimizethe impact of avian viral diseases on poultry. This project will also contribute to pandemic preparedness by evaluating the safety of candidate vaccine viruses in chickens for large-scale production in low-containment facilities, posing no adverse risk to animals, humans, or the environment.

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

This research aims to reduce the impact of major avian viral diseases on poultry production. The consequences and repercussions of these diseases on trade, food security, public health, and the livelihood of millions of farming and associated communities around the world are evident from the continued global prevalence and spread of avian viruses such as high pathogenicity AIV. The researchoutputs will include (i) improved knowledge of the factors that facilitate fitness, pathogenesis, and persistence of

AIVs in different avian hosts and the risk of zoonotic infection by AIVs; (ii) improved disease control tools (vaccines and antivirals) with greater ability to reduce the production losses, zoonotic and pandemic threats; and (iii) new data and publications leading to further improvement in disease control systems and animal welfare, (iv) professional development of the next generation of scientists and (v) socio-economic wellbeing.

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

Our research aims to understand how viral and host factors increase the transmission and disease severity of avian influenza viruses (AIVs). In the short-term, these findings will improve our fundamental knowledge of AIVs, which will aid in the development of disease control tools such as vaccines, antivirals, and probiotics. These tools will target multiple avian viral diseases affecting poultryand will provide strong and long-lasting immunity against AIV together with other major avian viral diseases.

In the mid-to-long term, we will develop more potent and efficacious disease control tools, reducing economic and welfare issues associated with administering multiple doses of viral vaccines to a singlebird. We aim to enhance vaccine potency and multivalency through novel approaches such as designing highly effective multivalent vaccines that can be delivered via mass delivery methods including vaccination to embryo in eggs before hatching, or via spray or drinking water. Our research also aims to develop novel antiviral therapeutics such as recombinant antiviral compounds that can also be administrated using the mass delivery methods, providing immediate protection against the target avian viruses.

Overall, this project will contribute to the reduction of poultry production losses, promoting global food security and improving animal welfare. This research will benefit various stakeholders in the poultry value chain, including commercial and backyard poultry farmers, animal and public health bodies, andthe veterinary product development economy. By providing direct benefits to farming communities andsubstantial indirect economic, public health, environmental, and social benefits, the effective control ofAIVs in poultry will also reduce their transmission to humans.

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

The research will advance our knowledge of the pathogenesis of AIVs, and of vaccines, antivirals, andprobiotics for improving controls against major avian viral diseases affecting poultry production.

Specifically, the project will generate a large volume of new data on molecular markers linked to AIVevolution, virulence, vaccine failure, and zoonotic infections.

The research will also inform new approaches to the development of improved multivalent vaccines fordifferent avian species (such as chickens, ducks, and turkeys) that can be exploited for other livestock and human diseases. The outputs of the project will be disseminated primarily via scientific publications and conference presentations. We are working on several collaborative projects with the poultry industry, and national, and international partners working to improve disease control systems for viral diseases of poultry and livestock. We will openly discuss and aim to publish “negative” data, which in regard to this project, involves the identification of viral and host genome factors and mechanisms which do not contribute to the pathogenesis of the disease, or that are not immunogenic determinants of AIV, and novel multivalent vaccines, antivirals or probiotics that do not provide protection.

The methods and reagents developed through this research will also be made available to scientists,veterinarians, and public health officials who are concerned with reducing the impact of infectious diseases affecting animal production and welfare, human health, and food security.

Species and numbers of animals expected to be used

- Domestic fowl (Gallus gallus domesticus): 2750
- Post-hatch and 500 embryos.
- Other birds: No answer provided

Predicted harms

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

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

This research programme involves the use of chickens, ducks, and turkeys, including embryos and post-hatched birds (neonate, juvenile, and adult). These birds at different stages of their developmentexperience disease outbreaks from the target avian viruses in the field and for which we want to improve disease control systems. There are no alternative less sentient model species available that provide the required assessment of AIV pathogenesis and identification of immune correlates of protection against target avian viruses.

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

The experimental procedures will involve:
Embryonated eggs will be used for assessment of the infectivity, replication, and virulence of AIV;for evaluation of the immunogenicity of subunit or recombinant vectored vaccines expressing antigens of AIV, and other important avian viral pathogens, or for evaluation of the efficacy of antivirals. The ageof the chicken embryos will be from 0-21 days. The age of turkey embryos will be from 0-28 days and duck embryos will be variable (0-28 days) depending on the specific breed of duck. Typically, a virus, vaccine, or antiviral will be inoculated into each egg via pipette tip or with a syringe into a small hole in the side of the egg made with either an ‘egg gun’ or a drill. Following inoculation, eggs will be incubated at 29 – 42^{O}C in a humidified rocking incubator or a warm room. Embryos will be monitored by candling (daily) during the appropriate incubation period required for the specific virus strain used inthe experiment (usually 72 hours post-inoculation). The embryos will be killed humanely one day before hatching for studies on the pathogenicity of AIV or will be allowed to hatch for studies on the immunogenicity and protective efficacy of vaccines, or for studies on antiviral efficacy.

Inoculation/vaccination of birds (chickens, turkeys, or ducks) with substances (virus, vaccine, antivirals, or immune modulators/enhancers or probiotics) via the appropriate route of administration. Birds will be closely monitored for any adverse effects (clinical signs) on their health on a regular basis(monitoring intervals will be adjusted according to the phenotypic characteristics of the virus strain or vaccine). Samples (swabs, blood) will be taken at intervals to analyse virus replication and/or immune responses. Swabs will be taken from oral and cloacal cavities, appropriate to species and challenge virus and

vaccine. The birds may be culled humanely at set intervals (post-vaccination and/or post-infection) to monitor host immune responses or to investigate the changes, presence, and distribution of the virus in infected animals. The birds may be kept up to 32 weeks post-vaccination, post-antiviral treatment, and/or virus challenge to assess the impact of the treatments. Since the challenge viruses will be pathogenic, emphasis will be placed on animal welfare and provisions have been made in the specific protocols.

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

Embryos in the final third of gestation may show signs of haemolysis of blood vessels, reduced or lackof movement. Embryos showing these signs will be killed immediately.

Birds (chickens, turkeys, or ducks) and mice will experience mild and transient pain associated withvaccination, blood sampling, or swabbing.

Following infection with virulent viruses, birds may develop clinical signs of disease depending on the virus strain. Clinical signs of disease may include weight loss, sneezing, coughing, ocular and nasal discharge or sitting alone, and reluctance to evade capture. Generally, the birds take about 72 hours torecover from these typical clinical disease signs. However, infection with some AIVs may result in death in a proportion of birds without any clinical signs appearing beforehand. The birds will be monitored for clinical disease signs at regular intervals so that the animal does not cross the defined severity limit for the virus strain used for 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 animaltype)?

- Mild more than 70%Moderate: 15-20%
- Severe or sudden death without clinal signs: 5-10%

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 October 2028

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 haveconsidered 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 no alternative less sentient model animal species available that provide the required in vivo animal models for investigation of virus-host interactions in the pathogenesis of and immune responses to avian viruses. Chickens, ducks, and turkeys were selected because they are natural hostspecies affected by different avian viruses (AIV, NDV, IBV, IBDV, FAdV, ARV, CAV, AMPV, ILTV) in the field. Therefore, we have chosen these avian species to model how AIV strains induce disease and persist in poultry. Similarly, vaccination studies ultimately require the establishment of immune correlates of protection for the target host species to be protected from disease. As there are differences in the immune systems between different avian species, it is therefore important to usetarget host species for the groups of viruses being studied here, where biological and antigenic variation is often related to, and dependent on the host of origin.

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

Where appropriate, cell culture and other relevant *in vitro* techniques such as *ex vivo* tracheal organ cultures (TOC) or embryonated eggs (up to 14-day-old embryos) will always be used as the initial methods for assessing virus infectivity and replication efficiency. Studies have indicated that the majority of avian-origin viruses prefer embryonated egg culture as a growth medium. Therefore, embryonated eggs may be used as an alternative to tissue culture. Culturing viruses in cells can allow mutations to develop in the surface glycoproteins altering the antigenicity and receptor binding preference of the virus. By using eggs as a growth media or investigating pathogenicity or host responses to infection, this vastly imitates a more natural infection with reduced selective pressure that can be exerted by tissue culture cell lines. The use of eggs is the efficient way to produce most avian viruses with no genetic changes altering virus behaviour and pathogenicity.

In vitro techniques including single-cell sequencing, and phage display techniques will be investigated for the generation of recombinant antibodies. Antibody sequences will be derived from immune cells (Bcells from blood or tissues) collected from naturally infected animals or animals used in other virus infection or vaccination studies.

Why were they not suitable?

There are no alternative less sentient model animal species available that provide the required virusphenotype (infectivity, tissue dissemination, and transmissibility protective efficacy) data for avian species against selected viruses.

A retrospective assessment of replacement will be due by 18 October 2028

The PPL holder will be required to disclose:

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

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that havebeen 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 statistician is consulted prior to each study to ensure that an appropriate number of animals is usedto generate meaningful results. The number of animals per group at each time point is selected to guarantee statistically relevant results for the assessment of protection and pathogenicity based on many years of experimental work on avian viral diseases.

Group size could vary depending on experimental design aspects such as non-infected controls, virusstrain or genotype, host species, challenge dose, immune status, and route of inoculation. However, studies usually involve groups of 6 to 10 birds.

The group size advised by the World Organisation for Animal Health (WOAH) will be adopted for testing the pathotype of avian influenza viruses known as the Intravenous Pathogenicity Index Test(IVPI).

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

We always consult relevant publications describing the sample size in pathogenicity and vaccination- challenge studies. We have taken into account the results from the many years of in vivo studies usingdifferent avian viruses that have been carried out previously. In addition, each animal study is reviewedby a statistician.

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

Pilot experiments will be undertaken in studies using viruses with unknown characteristics that differ in their infectivity and replication profiles in embryonated eggs in order to estimate an appropriate virus dose for productive infection in inoculated birds. The data from pilot experiments will be used for sample size estimation for follow-on virus infection, transmission, and vaccination experiments. We willcontinually review the published literature with a statistician to ensure the optimal number of birds for each experiment. Multiple studies will also be integrated in such a way as to utilise a minimum control group of experimental animals.

Samples such as post-mortem tissues can be shared between differentstudies and with other researchers.

In the studies that investigate the IVPI test of AIVCVV, the standard WOAH (World Organisation for Animal Health) advised procedure will be employed. For these tests, a minimum of 10 birds per grouphas been estimated to provide statistically valid data.

A retrospective assessment of reduction will be due by 18 October 2028

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything otherscan 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 welfarecosts (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 modelsand methods cause the least pain, suffering, distress, or lasting harm to the animals.

We are using the natural host (chicken, turkeys and ducks) for which we wish to contribute to betterdisease control against selected avian viruses. The experiments investigate the pathogenesis, transmission, vaccine and antiviral efficacy evaluations in chickens, turkeys or ducks. The precise number of groups will be dependent on the precise information required in each experiment.

Before in vivo studies, the infectivity, replication and virulence of candidate viruses will be examined in embryonated eggs of chicken, turkey or ducks. This will determine whether the virus is lethal to the embryo of the target avian species and provides some indication of the virus behaviour in vivo when we compare it to the strain that we have previous information about. Lethality will be monitored by candling of infected eggs, to look for the death of the embryo or indicators that the embryo should be euthanised (no movement of the embryo or blood vessel disruption). The mean death time from published work will always be considered when infecting eggs from known genetic and pathological backgrounds and the infected eggs will be euthanised at least three hours before this time. This meansthat virus-induced embryo death will be avoided. If the mean death time is not known, a pilot experiment using a minimum number of eggs will be undertaken to determine the approximate mean death time.

The harm to animals caused by procedures such as injection, swabbing and bleeding are mild and transient. The greatest harm to poultry will be the development of clinical signs of disease following infection with virulent viruses. In designing an experiment, close consideration will be given to the likelyseverity, ensuring moderate severity is not exceeded for a known virus (by administering a defined dose). In the case of viruses with unknown severity, a design will be applied which will ensure that clinical signs of disease will occur predominantly during normal working hours, thereby facilitating increased inspections (minimum of four times/day) and accurate assessment using a clinical score sheet. Overall, these measures should result in the severity limit not exceeding moderate. However, a number of experiments will require the use of the most virulent virus strains in order to produce scientifically valid results. However, the number of animals that may experience severe disease signs will be kept to a minimum. In some circumstances, animals infected with virulent viruses show little or no apparent clinical disease signs, and up to 20% per group may die unexpectedly. All unexpected deaths (regardless of virus challenge or not) will be investigated by post-mortem examination.

The birds used in this research will be housed either in open raised floor pens with solid floors which were specifically designed in consultation with our Animal Technicians and NACWOs or housed in negatively pressured poultry isolators which are designed to protect personnel and the environment from cross-contamination. Whilst isolators present inherent challenges, we are committed to providinghigh standards of animal welfare. The enrichment provided to all animals is a priority and this is no different for poultry in isolators. Birds are social animals and so they are housed in groups to allow fornormal social interaction. Where possible, they are also afforded more space than required within

the Home Office Code of Practice. Foraging, scratching, and pecking are all important behaviours to chickens and turkeys, so we provide our birds with substrate on the floor to allow foraging and dustbathing and toys to enable them to express their species-specific behaviour regardless of whetherthey are housed in open pens or in sealed isolators. Additionally, for ducks, plastic containers filled with water and a sandpit will be provided to enable them to express their natural water paddling/bathing behaviour.

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

There are no alternative less sentient model species available that provide the required immuno- pathological parameters or immune correlates of protection of avian viruses for target avian species. Where appropriate, cell culture, embryonated eggs (at immature life stage), and other relevant in vitrotechniques such as ex-vivo tracheal organ cultures will be used as the initial methods for assessing infectivity and replication efficiency of selected avian viruses.

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

Our procedures for determining pathogenicity, transmission, vaccine, and antiviral efficacy in avian species have been used for many years by us and others and have been published (e.g., where appropriate experimental designs include both positive and negative controls. Increased monitoring regimes when birds start to show clinical signs of disease). Further to refine the procedures, advice is taken from the Named Veterinary Surgeon (NVS). Pre-study meetings involving the researchers, Named Animal Care and Welfare Officers (NACWOs) and animal technicians will be held to discuss any advances in animal care. Meticulous records will be kept of behavioural, physiological, immunological, and virological measures in order to identify predictive markers and design humane endpoints for future experiments. Pain and distress scoring sheets specifically designed for each virus will be used. Highly trained animal technicians will monitor the animals as per experimental proceduresrequirements defined in the study protocols, ensuring they are comfortable and maximise their welfare status. All experiments will be followed by a wash-up meeting to discuss all aspects of the study and toensure lessons are learned.

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

We will adhere to the ARRIVE (Animal Research: Reporting of In vivo Experiments) guidelines. We willalso adopt the PREPARE (Planning Research and Experimental Procedures on Animals) principles. Inparticular, the allocation of birds to each study group will be random, and where possible observer biaswill be managed by blinding of treatment groups.

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

We will keep updated with published literature regarding animal experimentation in poultry and will regularly consult the NC3Rs website and available resources including guidelines, training materials, and practical information. We will maintain an open dialogue with the animal technicians and NACWOsat the establishment in relation to the enrichment that can be provided.

A retrospective assessment of refinement will be due by 18 October 2028

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 forfuture work of this kind?
- During the project, how did you minimise harm to the animals?

2. Mechanisms and Treatments of Neurological Disorders

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, ortheir effects, in man, animals or plants

Key words

Gene therapy, Epilepsy, Migraine, Neurons, Seizures

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

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 orscientific needs it's addressing.

What's the aim of this project?

We are working to understand how changes in the brain lead to diseases such as epilepsy and migraine. Once we understand what these changes are, we use this information to develop and testnew potential treatments for these diseases. Our work has resulted in several new gene therapy treatments that offer hope to patients with epilepsy who currently have no effective treatments.

A retrospective assessment of these aims will be due by 03 October 2028

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 advancedor 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 a computer crashes and you wish to fix it, it is necessary to figure out what went wrong - does it havea virus? Or does it just need its battery charged? What you need to do to fix the problem, is entirely dependent on knowing what went wrong and repairing the right thing. Replacing the battery won't fix a virus. Similarly, with diseases such as epilepsy, in order to develop treatments that work we need to understand what has gone wrong to cause the disease.

We are in a new phase of medical research, with gene therapy opening doors for treatments that can repair cells in our bodies in ways that were simply not possible a decade ago. Our group has been world-leaders in using careful animal studies to learn what has gone wrong in disease, and to harness the promise of gene therapies for repairing these problems to treat disorders of the brain. In order to deliver the promise of new gene therapies to the clinic, after we have developed potential treatments incells and other non-animal models, we need to confirm the treatments are safe and effective in the best available models of diseases to be sure our treatments do not harm patients and have the best chance of curing their symptoms.

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

We have been fortunate to have a many published outputs from our previous work, and based on ourexperience we anticipate this new project will provide:

Multiple new therapies for neurological disorders, offering hope to patients who currently have noeffective treatment options

Mechanistic and efficacy data that supports the transition of new treatments to first-in-human clinicaltrials.

A series of high profile, open access publications, detailing the promise and evidence of our potentialtreatments.

Presentations, including to physicians, patients and carers, and the general public explaining ourwork, and its potential clinical benefits.

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

Our ultimate goal is to offer new hope to many thousands of patients with severe drug resistant epilepsy, who currently have no effective treatment. We are preparing clinical trials now (our first trial isregistered on the database clinicaltrials.gov as NCT04601974), and hope to be treating patients with our first gene therapies by the end of this project. (First in human trials are planned to begin in 2023).

Our first-in-human trials will aim to offer less invasive, and better tolerated treatments to patientsapproved for surgery to remove the part of their brain that is causing epilepsy.

As well as gene therapies, in this project we are expanding to cell-based therapies, includingtherapies using cells collected from patients.

In the longer term, we believe our treatments may also be applicable to other diseases and disorders that are associated with altered activity in specific regions of the brain, this includes chronicpain, depression, anxiety and addiction, as well as other disorders, such as those that disrupt sleep and circadian rhythm.

The basic research underpinning this work will help us to understand how to treat different types ofepilepsy, and which other disorders might be safely and effectively treated with our approaches.

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

We have already established a spinout company which was established to maximise the ability of ourteam to bring our new treatments to first-in-human clinical trials. Our aim is that this company will develop a portfolio of gene therapy treatments that will allow us to give precision therapies to people with different types of epilepsy, and eventually, to people with other serious neurological disorders.

We also publish regularly in open access journals, and present at international congresses.

Several of us occupy international roles in leading bodies in the field (the International League AgainstEpilepsy, ILAE) allowing a platform to reach leaders in clinical and basic research.

Species and numbers of animals expected to be used

- Mice: 50000
- Rats: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, includingduration 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 small animals, mainly mice, but also rats. First, we confirm our potential treatments are safe and work as we expect in cells and cell lines (we use human cells derived from patients when they are available) prior to moving to animals). But in order to translate promising treatments to clinicaltrials, we need to test whether they also are safe and effective in the best models of disease available.Where possible, we use tissue collected from new born animals, which when grown in vitro can be used to carry out multiple tests in parallel with minimal suffering.

However, there is growing appreciation from patients and carers that treating diseases

such as epilepsy involves more than just dampening excess brain activity. We also need to ensure other symptoms of these diseases (known as co-morbidities) are safely and effectively treated by our interventions. For this we need to use adult animals that have the symptoms of the disease (e.g. seizures), and are able to carry out behaviours such as learning simple mazes, or exploring a newspace, which can inform whether they are also experiencing anxiety or learning disabilities.

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

We aim first to establish the effectiveness of our treatments in isolated cells, so that as few animals aspossible can be used to test the largest number of treatments. For these tests neonatal animals are killed as quickly and painlessly as possible in order to collect brain tissues, which can then be grown invitro. Where possible we also test treatments in human derived cells (known as iPSCs, or induced Pluripotent Stem Cells).

Only after we are confident our treatments work in these isolated cells, do we carry out tests in living animals. This involves breeding animals with known genetic characteristics or symptoms (sometimes including epilepsy). We use surgery in some animals to implant small devices that allow us to record their brain activity - so we know if they are having seizures. We also use specific treatments to cause animals to develop seizures, similar to how some people develop seizures after an injury. Only when animals have established seizures, similar to the experience of the patients who we are seeking to treat, do we carry out an injection to deliver our treatment. In some cases this involves an additional surgery to inject directly to the part of the brain causing seizures (this is how we plan to treat patients).We then observe the animals carefully, usually for several weeks, to see if their seizures go away. Wealso carry out a series of behavioural studies to investigate whether the animals are feeling anxiety, if they are able to learn simple mazes, and whether the treatment has allowed them to return to normal activities. Mice are typically studied for 26 weeks or less, but rats may be kept for longer, sometimes up to two years, to be sure our treatments remain effective for a long time without fading away, as wehope our patients will only need to be treated once.

A final branch of our work, which underpins all our new treatments, is understanding what is causing the symptoms of the diseases in the first place. For this work, we use animals, sometimes carrying genetic mutations that cause seizures, or which allow us to see neuronal activity. We may induce chronic or acute seizures using specific treatments. We may also open a window in the skull to allow us to deliver treatments and record the activity of the brain, to ask how the seizures are caused, and whether our treatments are able to stop seizure activity in a safe way. For these experiments mice willtypically not be kept for more than 26 weeks.

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

We do all we can to minimise suffering, but in order to test the efficacy of our treatments against seizures, it is necessary that animals do experience the sorts of seizures we wish to treat in patients. Patients report that seizures are not painful, but that afterwards they are tired, and if they fall, they maybe injured. Increasingly, we are able to develop treatments that reduce these seizures in animals over a few weeks, but our 'control' animals can continue to have seizures for the duration of the study, whichis usually less than 12 weeks for mice and only occasionally longer for rats (when we need to ensure that the treatment remains effective for a long time - sometimes more than a year).

As many of our procedures do involve surgery, and some animals do not react well to surgery, we dooccasionally see some weight loss. Weight loss is usually transient, and once animals recover, they put weight back on. Sometimes animals pick at their sutures as well, and can even re-open a suturedincision. Typically this sign of irritation and itching goes away after a few days, as the incision heals.

Some animals are born with mutations that cause symptoms such as seizures. These animals may experience these symptoms throughout their lives. In most cases this does not affect growth or activity,but in a few genetically modified strains the symptoms are more serious, as in the human disease, andcan lead to a proportion of the animals experiencing Sudden Unexpected Eeath in EPilepsy (SUDEP). An important part of our planned work is to reduce these symptoms in these important lines of mice that model human diseases that need treatments. We think it may be possible to rear these mice in such a way that they are healthier and suffer less SUDEP. If this is possible it will impact many laboratories that work with these mice and could also make sure their animals suffer less.

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 many animals they are experience no real harm. They live in their cages and breed. Of an estimated total of ~50000 mice, approximately 61% will have mild or "breeding only" experiences. About 22% will experience moderate severity, mainly due to surgery or clinical symptomsof the diseases we are trying to treat. About 17% will be newborn animals that are killed very quickly bya humane method so we can collect tissues. The remaining ~0.5 to 1% (or estimated <400 animals) will experience severe outcomes, many of these will be the animals modelling the human epilepsy, Dravet syndrome and a small percentage of animals with chronic epilepsy. These animals experience Sudden Unexpected Death in EPilepsy (SUDEP), where they are found dead with no clear reason why - a phenomenon we are trying to understand to help patients.

For rats (estimated <3000), ~55% will be newborn animals killed quickly and humanely to collect tissue. Approximately 39% will be moderate, either due to surgery or clinical signs (typically seizures).Unfortunately, as with mice (and human patients) in rats with epilepsy we do see SUDEP, but we expect this to affect fewer than 0.5% in total.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 03 October 2028

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animalswere 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 main aim is to develop new treatments for neurological diseases. Much of our preliminary work isdone in cells and cell lines, but ultimately in order to show the treatments are effective on symptoms such as those experienced by patients with epilepsy, we need to work with animals that have these symptoms. Where possible we use human-derived cells, including induced Pluripotent Stem Cells (knowns as iPSCs), to determine how diseases might affect single cells, but in order to understand seizures we need to use live animals that can experience seizures. We have worked, where possible, with models in fruit flies who share many genes with humans, but many of our treatments are at an advanced state of moving to first-in-human clinical trials, and before we are allowed to test these treatments in human patients we need to be convinced they work in mammals that share the same types of neurons in their brains as humans have. Once we have data showing our approaches work, we need to carry out safety studies and tests at different doses to present to the regulatory bodies before we can get permission to carry out clinical trials. These safety and dosing data also rely upon animal studies.

Another important concern comes from patients and carers who are telling us that challenges with behaviour and memory are also very important to their well-being, and we are working to be able to test whether our treatments work to help behaviours such as memory and anxiety. We need to carry out studies in living animals, including animals with seizures or other symptoms in order to measure ifour treatments can help with these behaviours.

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

We only use live animals after we have exhausted the possibilities of cells and cell lines. Before we test treatments in vivo, we use cell lines and cultured cells to screen our treatments. This includes, where possible, induced Pluripotent Stem Cells (iPSCs) to validate our treatments as much as possiblebefore moving to animal cells.

Why were they not suitable?

We find cells and cell lines are suitable for understanding many aspects of how a treatment works, andwe do a substantial amount of work to understand our treatments and the causes of disease in cells and cell lines before using any animals.

However, in order to test for whether a new treatment can stop a neurological disorder, we need to useanimals that have the same types of neurons as humans do. Studies in cells or networks of cells grownin Petri dishes can only assess whether our treatments work at the cellular level, or in simple networks - which is very useful for knowing whether to continue our studies of a treatment, but isn't enough information to risk injecting a new treatment into a patient. Ultimately in order to be confident enough todeliver what may be an invasive and permanent treatment to a patient's brain, we need to be very confident that the treatment is safe and effective, and testing in living, behaving animals is the only waywe have to be confident that our treatment can stop symptoms that involve networks within the brain.

A retrospective assessment of replacement will be due by 03 October 2028

The PPL holder will be required to disclose:

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

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that havebeen 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 estimating our future numbers based on more-or-less continuing to use the same numbers ofanimals in the future as we have in the previous project licence (PPL) which supported the same sort of research. We will likely grow as a group (currently ~30 researchers collaborate and share expertiseon this PPL), but we are getting better at using fewer animals, particularly in breeding.

It is difficult to estimate the total numbers of animals, as our use depends on how successful we are,and how many charities, governmental bodies and industries offer to fund our work. We have severalbig programmes funded by the government and charities, and as we find new promising treatments, we will also be applying for more funding for additional studies to help bring them to patients.

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

For all our new treatments we use a study design that is powered to give clinically meaningful effects with the smallest number of animals. Where possible we use animals as their own controls, as this canreduce the number of animals needed to see a meaningful effect.

We also use the Experimental Design Assistant (EDA) when planning new studies.

We are working to reduce animals needed for breeding, including using timed matings and other stepsto allow for smaller numbers of animals born.

Where possible we share tissue, so that fewer animals are needed.

We are working with colleagues at other institutions to develop a long-term experiments that will allowus to use sensors that can collect far more data from each animal than our current equipment.

Ultimately we hope that the greater amount of data from each animal may allow us to reduce thenumber of animals we need to use.

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

We are working with our Animal Welfare and Ethical Review Body (AWERB) and our technical staff to reduce animal numbers in breeding protocols. This involves using timed breeding, and working to keepabreast and train the researchers (Personal licence holders, or PILh) who manage colonies in best practices in order to reduce numbers as much as possible and prevent animals from being bred that are not needed for experiments. We also have established a default 'do not wean' that ensures litters that are born are not weaned unless needed for experiments (some animals must be bred regularly to maintain their ability to rear young).

We have instituted a strategy of having pilot studies, typically first of individual animals, then of smallgroups of 3, for any new treatment or intervention to ensure it is safe and well-tolerated before beingtrialled on a larger group. This reduces the chance of a large group of animals entering a study for atreatment that is ineffective, or has adverse effects.

We routinely carry out our studies in a blinded, randomised design so that we reduce bias. This meanswe are less likely to think a treatment is effective when it is not, and therefore less likely to keep testingit in additional models.

A retrospective assessment of reduction will be due by 03 October 2028

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything otherscan 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 welfarecosts (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 modelsand methods cause the least pain, suffering, distress, or lasting harm to the animals.

We use genetic and induced models of neurological disorders, particularly epilepsy, but also other disorders that affect neuronal activity in ways that might allow us to develop new treatments. Where possible we maintain any genetic lines for which animals have symptoms as heterozygotes, i.e. theyhave one copy of the mutated gene and one copy of the healthy gene. This means most of our breeding animals do not suffer any symptoms. The vast majority of animals used for breeding experience no symptoms.

We have worked with these models of neurological disorders for many years, and have refined them toreduce suffering and distress. Over the course of our studies we have refined surgeries, so that very few animals have adverse effects, and new surgeries are piloted in individual animals and then small groups to be sure the new methods are well-tolerated.

We collaborate widely in epilepsy research, and implement changes reported by any research groupsin the field that refine our models. However, in order to show treatments are effective at reducing symptoms, it is unavoidable that animals do have to experience the symptoms when untreated (we hope the animals that are treated will have many fewer or no symptoms, of course).

We are constantly updating our models of epilepsy to find those which are most tolerated by animals,but which are also most relevant to human disease so that we are most confident our new treatmentswill translate to human patients.

We do have multiple models of epilepsy, including some which are more serious. This is because thereis a risk that if a treatment is only shown to be effective in a single model of epilepsy it may not translate to be an effective treatment in human patients. Therefore the gold standard in the field is to test treatments in more than one model of a disease, ideally with different mechanisms. We aim to show each of our treatments is effective in at least two different models.

We do have one model of a human disease called Dravet syndrome, which is a severe childhood epilepsy. Children with Dravet can be very ill, and can die of Sudden Unexpected Death in EPilepsy(SUDEP). Mice carrying the same type of mutation also can have many seizures, and unfortunatelythey also experience SUDEP, often at about the time when they are weaned from their mums.

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

Where possible we do use neonatal animals to collect tissues to carry out studies in vitro. Only whenwe are confident our approaches work in vitro using these immature tissues do we progress to using mature animals. We also test in cell lines and human derived cells that can model different cell types,including neurons (these are called induced Pluripotent Stem Cells or iPSCs).

We collaborate with groups that use less sentient models, including slime molds and fruit flies, and in some cases have had very important insights into the mechanisms and treatments of diseases from these organisms. However many of the epilepsies we wish to treat are associated with brain structuresthat do not exist in these less sentient organisms, so they cannot be well-studied or treated in these organisms. Also some of our treatments target types of brain cells that don't exist in fish or fruitflies, sowe cannot test them in these organisms.

Where possible we do use animals under terminal anaesthesia for some studies. However anaesthesia interferes with brain activity, and many of our studies rely upon knowing whether a treatment changes brain activity, so it is necessary to study these treatments in animals that areawake.

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

We regularly monitor animals after any surgical procedure to ensure they are recovering well. We provide wet food, and when animals show signs of weight loss we have a food pantry of treats to helpencourage animals to eat (Nutella is a favourite).

We regularly consult with our Named Vetinary Surgeon (NVS) to ensure our pain

management is asup-to-date and effective as possible.

Working with our Biological Services (BSU) staff who look after our animal facilities, we work to provideall animals with safe and fun enrichment, including running wheels (mice only), houses, tunnels and bedding.

Some animals with epilepsy or implanted devices need to be housed individually. We have worked to introduce periodic social sessions, and cages with transparent dividers so animals can interact with peers even whilst housed separately. Many animals with epilepsy are monitored with 24/7 videoing aswell as recordings of their brain activity (EEG), which can indicate if number of seizures is increasing, or if individual seizures are prolonged.

We are also interrogating new refinements, such as treating animals born with genetic mutations thatcause epilepsy with 'best practice' treatments informed by clinical studies in children with these samegenetic epilepsies. We hope this will make animals with these mutations have fewer seizures or othersymptoms.

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

Local Named Vetinary Surgeon (NVS) and our Named Animal Care and Welfare Officer (NACWO) areour first points of contact for queries.

We also use 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

Local guidance on food and water control

For pre-clinical studies we follow guidance given by the Medicines and Healthcare products RegulatoryAgency (MHRA) on animal numbers and time points, as well as updates posted on our institutional website.

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

The Researcher leading this project (the Project licence holder, or PPLh) is the Chair of the local Animal Welfare and Ethical Review Body (or AWERB) which has representation by the NC3Rs representative. The AWERB has a regular and strong emphasis on collecting and sharing best practices from across the institution and elsewhere, with an aim of identifying and promoting any newapproaches that advance 3Rs (Replacement, Reduction and Refinement) in any area of research involving animals.

A retrospective assessment of refinement will be due by 03 October 2028

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 forfuture work of this kind?
- During the project, how did you minimise harm to the animals?

3. Neural, Physiological and Behavioural Mechanisms Underlying Cognition and Emotion

Project duration

5 years 0 months

Project purpose

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

Key words

Emotion, Cognition, Mental health disorders, Brain Networks, Treatments

Animal types	Life stages
Marmosets	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.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Uses non-human primates

Objectives and benefits

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

What's the aim of this project?

This project aims to identify how different parts of our brain control our ability to learn, remember, plan, attend, display positive and negative emotions and socially interact with others. We aim to understand how these brain regions work together or individually to provide us with these abilities, how stress and genes may affect how they function, how this may lead to mental illness and how current treatments formental illness work in the brain.

A retrospective assessment of these aims will be due by 14 October 2028
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 advancedor 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?

Disruption of these various aspects of our behaviour (cognitive, emotional and social) are associated with a wide range of mental health disorders (such as obsessive-compulsive disorder (OCD), depression and anxiety) but also in natural aging. These disruptions combine to produce symptoms which are only successfully treated by current therapies in some people but not others. We also do notunderstand how they work when they are successful and so everyone gets given the same treatment in order to find out whether it will work or not. We cannot decide in advance who will be treated successfully. To move forward from this situation, it is essential we gain a basic understanding of how individual brain regions underlie the cognitive/emotional/social disruptions which create these symptoms. This issue can only be addressed in experiments with animals, in which specific, localised manipulations can be made and their effects on behaviour determined. This provides information not only on what brain regions are responsible for our cognitive, emotional and social behaviour, but also how stress and specific chemicals and genes interact with them to cause their disruption that leads to mental health disorders.

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

The results from studies performed in this project will determine the key brain regions that, if dysregulated, may underlie the different symptoms associated with disorders of cognition and emotion.As a consequence it will provide us with greater insight into the nature of clinically relevant symptoms which will help to identify groups of patients that may be better treated by particular drugs. Such a knowledge base would greatly improve clinical outcomes.

Understanding the biological (genetic) and non-biological (environmental) bases of individual differences in emotion and cognition will enable us to gain insight into both vulnerability to mentalhealth disorders and also to differences in responsivity to drug treatments.

In addition, by characterising the development of brain circuits and determining the different time-pointsat which distinct circuits undergo the most change during development, and the effects of perturbation at these different time points, we will also provide further insight into the aetiology of psychiatric disorders and again help stratify patients more effectively.

We will be able to define the neural and neurochemical mechanisms by which current drugs work, which combined with better knowledge of patient symptoms, will help us to target their use in individualsubjects more effectively.

Finally, we will gain a detailed understanding of the marmoset brain anatomy and connectivity that willserve as a very valuable informational framework for many neuroscience researchers in both the present and future.

Publications will be an important part of the output for all information gained throughout the duration ofthe project.

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

The intended overall benefit of this research will be directed to society as the intended increased understanding of the biobehavioural basis of cognitive and emotional symptoms of neuropsychiatricdisorders will have far reaching social and economic implications.

The first beneficiaries of this research will be the scientific and clinical communities. The publication in journals with Open Access, which, for basic research, remains the most effective route to communicateresearch findings, allows the research findings to gain international impact and prominence, relatively quickly.

The second beneficiaries would be the patients themselves as the output of this research will help provide insight into the varied neural dysfunctions that can underlie the range of cognitive and emotional symptoms associated with neuropsychiatric disorders, guiding new treatment strategies as well as providing insight into the mechanisms by which current therapies have their efficacious actions.

We also intend to impact pharmaceutical company policy as they return to the development of medications for the symptoms of neuropsychiatric disorders. We recently collaborated with a pharmaceutical company to consider new drug targets for anhedonia, the loss of pleasure, a prominentsymptom of several psychiatric disorders.

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

We communicate our research as widely as possible to academic and scientific communities. The outcome of our research will be published and disseminated to the scientific community in both high- impact scientific journals with Open Access and at national and international meetings and summer schools. Our results will be targeted for publication in peer-reviewed high impact journals (e.g., Science, Nature Neuroscience, Neuron, Proceedings of the National Academy of Sciences, BiologicalPsychiatry, Neuropsychopharmacology, Cerebral Cortex, Journal of Neuroscience). We have a strong track record in maximising the output of our work, including both positive and negative results.

Manuscripts accepted for publication will be made open-access and archived in an institutional repository to ensure the widest possible accessibility and impact of our work, thus meeting the new HEFCE policy on peer-reviewed articles and conference proceedings. We will also continue to publishconceptual papers and reviews which are often highly cited and increase the profile of our work and the field in general.

Our results will be disseminated through presentations (plenary lectures, symposium lectures, posterpresentations) at international and national conferences and summer schools.

We also communicate our research to non-scientific communities. The publication in journals with Open Access will only be the first step in a wider dissemination and communication strategy aiming to immediately increase our impact on the general public. We have already published an article in The Conversation in 2021, a magazine that specialises in presenting scientific results in an easily digestibleformat for the public. We

have also had our papers reviewed in other USA scientific outlets for the public including techexplorist, neurosciencenews and scitechdaily. Thus, we will rely on publicisation ofour work by local and national media groups.

Finally, we communicate our research as widely as possible to clinically active scientists and clinicians.I am an elected fellow of the Academy of Medical Sciences which facilitates my interaction with clinicians and recently have been invite to give a presentation at the Academy of Medical Sciences in the USA. We aim to highlight how our findings impact not only our understanding of the aetiology of the symptoms found in psychiatric conditions but also insight into strategies for their treatment.

Species and numbers of animals expected to be used

- Marmosets: 300

Predicted Harms

Typical procedures done to animals, for example injections or surgical procedures, includingduration 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 neural mechanisms underlying cognition and emotion requires a freely moving, behaving animal. It is not possible to investigate behavioural functions of the brain in a simplified experimental setting such as a tissue culture or in an artificial and biologically unrealistic computer simulation. Existing techniques used in humans, such as brain imaging are not only limited in spatial, temporal and neurochemical resolution, but also provide purely correlative information on structure- function relationships which does not address the crucial issue of the causal involvement of particular regions in specific psychological processes. While studies of patients with neurological damage can provide causative information they fail to provide neural or neurochemical specificity as the damage is non-specific to particular brain structures. Since there is abundant evidence that the normal functioningof much of the brain is comparable across species, not only structurally but also functionally, including the behaviour it supports, this facilitates the extrapolation of findings.

We have chosen to work with a new world monkey, the common marmoset. The marmoset brain, especially the cerebral cortex has an organisation far more similar to humans than that of rodents, the latter species most commonly used in brain studies. The cognitive and emotional functions under studyare poorly developed in rodents and this is reflected in their poorly developed brains compared to humans. The brain regions known to be involved in complex behaviour in monkeys and humans, e.g. the prefrontal cortex, are markedly reduced in rodents. Specifically, whilst the cortex makes up 80% of the brain in humans it makes up only 42% of the rat brain and the overall structural organisation of the rodent prefrontal cortex is not comparable. The prefrontal cortex in other species such as pigs and sheep is almost completely unknown, as are these animal's cognitive abilities and social and emotionalcapacities. Such higher-order capacities are very much linked with the complex social interactions that are the hallmark of most primates, hence why we have chosen the marmoset.

We are studying the brain not only in adulthood but also in development because many of the psychiatric disorders have their onset during development. Therefore, we need to

understand the development of these highly developed brain regions in order to understand how they may go awryand lead to neuropsychiatric symptoms.

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

Marmosets used in this project will be exposed to several sets of behavioural and experimental procedures the cumulative severity of which never extends beyond moderate severity. A typical study lasts between 12-24 months during which time an animal may receive approx. 5 or 6 anaesthetics, only 2-3 of which will be for surgical procedures, the rest for restraint purposes only, e.g. neuroimaging.Some animals will only receive procedures as adults whilst others will receive procedures as juveniles.

All animals will first receive short, behavioural tests in the home cage that last no more than 20 mins, to determine their trait anxiety. The tests include measuring their responsivity to an unknown human that stands in front of their cage for 2 minutes and to a rubber snake placed in a box that sits on the floor of their home cage for 5 minutes. This is important to allow animals to be directed into studies thatthey are most appropriate for.

The various behavioural procedures marmosets will be exposed to aim to assess their individual performance in tasks that measure a range of cognitive and emotional functions including attention, behavioural flexibility, decision making, working memory, long term memory, social cognition, interest inreward and sensitivity to mild threatening stimuli (such as darkness, intermittent white noise, unknown humans, rubber snakes). In most of these tests, visual stimuli are presented on the touchscreen and the monkey makes a voluntary response to one of the presented stimuli. Testing in these tasks typicallyrequires animals to be temporarily sequestered into a specialised testing apparatus. This testing also sometimes requires marmosets to have their water access restricted or to reduce the amount of 'sweet' foods they get so the juice rewards in the test are more valuable to them. Behavioural tests typicallyoccur daily, never last more than 40 minutes in a session and only take place Monday to Friday.
Typically, they have weekends off.

In some cases, marmosets (adult only) will also have their heart rate and blood pressure monitoredalongside the behaviour if we are studying emotional reactivity. In this case they receive a one-off surgical procedure which implants a telemetry receiver into a blood vessel (entire surgery takes 1.5hours and return to home cage within an additional hour and a half) allowing for the remote measurement subsequently of their cardiovascular activity when they are freely moving.

In many cases adult marmosets also receive brain surgery involving: (i) infusion of viruses (e.g. AAV derived) into a specific region of the brain that enable the insertion of a designer receptor into specific neurons. These receptors are inert unless the animal subsequently receives a designer drug that specifically targets the designer receptors but has no other targets in the brain. (ii) insertion of tiny metal tubes (called cannulae) into the brain to allow subsequent infusion of substances into the targeted region whilst awake or (iii) infusion of a compound that has a permanent or longer term effect(1 week - permanent) in the brain. In some cases these procedures will be performed on juveniles at 10 months of age or older but in these cases (i) or (iii) are the preferred options as they are the least invasive.

Subsequently, having received i or ii, adult or juvenile marmosets will receive drugs peripherally (typically 20-48 injections) or through the implanted cannulae (up to 2-3 times within a week, typically 20-30 infusions in total per region) to temporarily change the activity in the specific brain region and itseffects on behaviour and/or blood pressure/heart

rate (adult only) measured.

Some marmosets (adult or juvenile) will undergo brain imaging (typically lasting 90 mins and 4 scans intotal) to help assess the effect of the brain manipulation. Often the scans will be performed both beforeand after the procedure so that the brain can be compared at these distinct time periods, allowing marmosets to act as their own control. Here, an animal receives anaesthesia for restraint purposes only.

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

Overall, the incidence of adverse events is expected to be low, with the majority of procedures not anticipated to produce adverse effects. This is a result of the many steps taken to ensure best practice and to mitigate adverse impacts. Everything will be done to limit the pain, suffering, distress and lastingharm to the animals within our care at every opportunity and for every procedure. Nowhere in the project is it expected that animals show clinical signs of ill health, and they are checked routinely in case any such signs emerge.

Most adverse effects expected relate to the initial acute recovery phase following surgery, whereby complications may arise from the procedure itself (e.g. localised facial swelling) or the prolonged use ofanaesthesia (e.g. protracted recovery to normal behaviour). Such effects typically resolve within 3 hours but can extend to approximately 24 hours. With employment of best practice treatments (e.g. fullanalgesic regimen) and extensive monitoring the overall impact of surgery to the animal is limited as much as possible. The acute phase following a surgical procedure involves the animal being actively monitored very closely for any signs of deviation from the normal recovery process. Additionally, extra care is taken during the first week after surgery to observe any changes in normal behaviour or appearance. Long term implant sites are cleaned regularly throughout the life of the animal to preventinfection, and the cage furniture altered to minimise environmental hazards.

Behavioural testing, and the testing apparatus, are highly habituated and do not produce adverse effects apart from transient mild anxiety. However the access to water in the home cage may need to be restricted during the more intellectually demanding experiments utilising a liquid reward. This waterrestriction only limits the time that anmimals have access to water, not the volume but has the capacityto impact the animal’s general well-being. This is vigilantly watched for but rarely observed. Water restriction does not affect the weight of the animals, who often ignore the water when it is first returnedto their cage, suggesting that they are not very thirsty.

Animals may experience transient discomfort when being handled by an experimenter and removed from their home cage, such as is necessary for maintenance of surgical implants, or administration of substances. Discomfort associated with handling would only last a few minutes. However, they are habituated to this process over a period of time to ensure they are not stressed by the procedure and thus are not expected to experience much discomfort at all. They normally acclimate to this process quite quickly and are frequently rewarded with treats such as a small bit of marshmallow. Peripheral injections themselves can produce mild discomfort (i.e. slight bruising at leg injection site). In such a case with intramuscular injections, legs are alternated to minimise effects on the muscle and they are checked by the Vet at regular intervals (after every 12 injections) with approval required before anymore injections can be performed. Animals are watched very closely for adverse reactions i.e. tremoring, after all drug treatments, and we have robust

protocols to alleviate such reactions if they dooccur.

Animals may experience transient discomfort as a result of brain manipulations or peripheral physiological challenges (i.e. hormones) that alter their internal bodily and emotional states. Sucheffects are anticipated to produce only very mild impacts on an animal's well being with durationssubject to the substance used but typically no more than 2 hours, usually much shorter.

Given the cumulative nature of adverse effects we do everything we can to limit the number of adverse effects experienced across their lifetime. This includes examining closely the transition between any procedures, especially if the animal has experienced any adverse effect or negativeimpact from a 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 animaltype)?

- Mild 10%
- Moderate 90%

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 14 October 2028

The PPL holder will be required to disclose:

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

Replacement

State what non-animal alternatives are available in this field, which alternatives you haveconsidered 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 neural mechanisms underlying cognition and emotion requires a freely moving, behaving animal. Existing techniques used in humans, such as brain imaging are not only limited in spatial, temporal and neurochemical resolution, but also provide purely correlative information on structure-function relationships which does not address the crucial issue of the causal involvement ofparticular regions in specific psychological processes. While studies of patients with neurological damage can provide causative information they fail to provide neural or neurochemical specificity.

Thus, this research requires the use of animals engaged in specific behavioural and cognitive tasks.

There is abundant evidence that the normal functioning of many of these neural systems is comparable across species, thus allowing a certain amount of extrapolation of findings across species.Some ex vivo techniques will be used to complement the in vivo studies presented. DNA, RNA and protein analyses will be performed on blood and brain tissue obtained pre- and post-mortem, respectively. The brains of all animals receiving brain manipulations will be analysed post-mortem to locate the positions of cannulae and/or lesions in the brain. This will inform future surgeries and refine surgical procedures.

It is essential to use an animal model with cognitive and emotional functions translatable to/similar to those found in humans. These functions are poorly developed in the rat which is reflected in the organisation of their brain. The anatomical features of those regions of the brain known to be involved in complex cognitive and emotional behaviour in monkeys and humans, e.g. prefrontal and temporal association cortex, are markedly reduced in rodents, Specifically, whilst the cortex makes up 80% of the brain in humans it is only 42% of the rat brain and the overall structural organisation of the rodent prefrontal cortex is not comparable. The prefrontal organisation of other species such as pigs and sheep is almost completely unknown, as is their cognitive abilities and their socio-emotional capacities. These complex behaviours are linked to the complex social societies that primates live in including marmosets, which is the species of choice for this work.

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

Tissue cultures (including brain organoids) and artificial computer simulations.

Why were they not suitable?

Tissue cultures (including brain organoids) are unable to contribute to a functional, behaving circuit, thus cannot perform cognitive tasks and do not express emotion whilst artificial computer simulationsare biologically unrealistic.

A retrospective assessment of replacement will be due by 14 October 2028

The PPL holder will be required to disclose:

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

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that havebeen 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 deciding on group size a number of factors are taken into account. My research group has extensiveexperience of publishing experiments on marmosets in high-impact journals with rigorous statistical peer review. We use the ‘appropriate number (n) of marmosets compatible with adequate statistical power for hypothesis testing, according to the

"Reduction" principle from the 3Rs. The precise number used in groups has varied from study to study (from sample sizes of 3-8) depending upon prior knowledge about inter-individual variation in (i) performance of animals on the particular task and (ii) the effects of the neural manipulation, both of which affect the anticipated effect size. Smaller sample sizes (3-6) have been used in the more recent years because of our refinements to our subject study subject allocation, behavioural training and surgical interventions, which have led to enhanced effect sizes. When we embark on a new study we tend to start off with 2 lead animals in which we test out our hypothesis. We are looking for large obvious effects e.g. the abolition of anticipatory cardiovascularand behavioural arousal in an anhedonia (loss of pleasure) study, which can be seen visually in individual animals. Where possible these lead animals will be incorporated into the main study if few, ifany, changes have to be made in the experimental design before the rest of the animals enter the study. It should be stressed that our effect sizes tend to be large because we have strong hypotheses based often on previous findings from our own lab and by having tight control over experimental variables. Based on our recently published and unpublished experimental manipulation studies, we have planned for group sizes of between 5-8 in the majority of our studies.

Appropriate numbers for the study of genetic polymorphisms are still being clarified. We found an n=8group size to be sufficient to identify statistical differences in pharmacological responses and brain neurochemical and structure differences whilst molecular studies have required n=3 per group and behavioural observations needed n=15.

Similarly appropriate numbers for the study of the brain and behaviour over development are still beingclarified. This work is unprecedented and so the estimation of numbers is based on findings from our own earlier developmental imaging study in marmosets as the clearest indicator of the kinds of effect sizes and background variability we might expect.

We are familiar with the PREPARE and ARRIVE guidelines (https://proecopa.no/prepare; https://arriveguidelines.org) and will ensure all our experiments are designed in adherence to theseguidelines.

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

using a carefully controlled behavioural testing apparatus so that the particular cognitive abilitiesunder study can be studied in isolation which helps reduce variation in performance.

using structural MRI to target certain brain structures to ensure that the brain manipulation iseffective in the majority of animals.

using animals wherever possible as their own controls to increase the power of statistical comparisons, minimise variability, and minimise the number of animals used

when separate controls are required, not necessarily matching the number of controls to the number of experimental animals but still ensuring they are sufficiently balanced to ensure statistical power

screening to ensure suitability of animal for particular study, thus also minimising variability.

re-using animals when they suffered no significant adverse effects during or as a result of their previous use. Previous use will not prejudice the outcome of the study on which they

are reused, and after the completion of the previous procedure and before the intended reuse the NVS has determinedthat they may be kept alive and that their health status and condition is compatible with proposed reuse in compliance with ASPA requirements. Re-use will not take place if an animal has received e.g.intervention surgery but an animal is re-used if all they have received is e.g. non-invasive imaging of their brain during development or peripheral drug injections/blood sampling.

repairing surgical implants whenever possible. Simple repairs occur whilst the animal is awake and minimally restrained by an experienced holder, whereas more complex repairs occur under anaesthesia. Since the repair procedure occurs to an implant rather than the animal itself, repairs arepainless. However, if anaesthesia is required for more complex repairs, analgesia will also be administered. Therefore, the cost/harm of repairing a cannula on an already trained and cannulated animal outweighs the cost/harm of needing to replace the animal in the study.

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

Using pilot studies to optimise the parameters of a brain manipulation or experimental procedure and to determine the dosage of peripherally administered drugs to elicit subtle behavioural changes before running the main study.

A retrospective assessment of reduction will be due by 14 October 2028

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything otherscan 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 welfarecosts (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 modelsand methods cause the least pain, suffering, distress, or lasting harm to the animals.

Marmosets are a particularly valuable species to use for our work as their relatively small primate brainmakes it possible to target cortical and subcortical structures and make regionally selective changes inthe brain with relative ease, with minimal risk to the animal.

We use a wide array of methods in our research which have been, and continue to be, optimised toensure least pain, suffering, distress, or lasting harm to the animals.

The behavioural tasks we use are designed to be similar to those used to test intact and brain damaged humans, maximising the ability to extrapolate findings from our marmoset studies directly into the clinical setting. To avoid unnecessary stress associated with behavioural testing, our animalsare (i) trained to voluntarily get into a carry box in the

home cage to go to the test apparatus (ii) can move freely once in the test apparatus and (iii) are tested for less than 40 minutes.

Water restriction for behavioural testing is only used when an animal's responses are variable in order to provide additional motivation to ensure stable and high performance across sessions. Access to water is initially only restricted for a few hours immediately before testing. If needed, some animals may then move on to longer periods of restriction, only having access to water for two hours at the endof the day to have the desired effect on their performance. This restriction only induces mild thirst, not dehydration, and to limit stress, the animals have two days of uninterrupted access to water every week, and have a break from restriction for at least a week every six months.

Systemic administration of biologically active chemicals (e.g. drugs) either through experimenter- delivered injections, or via previously implanted subcutaneous minipumps. Dosing procedures will beundertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm and are the minimum number of procedures (adults and juveniles) consistent with the objectives that will minimise the already transient, mild discomfort.

For blood sampling procedures no more than 10% of total blood volume on one single occasion and15% of total blood volume in any 28-day period will be taken following established guidelines for the marmoset (General principles NC3Rs).

Implantation of inert cannulae into the brain under anaesthesia allows for subsequent infusion of compounds through the cannulae to manipulate brain activity in localised regions and pathways (adult and juvenile) temporarily. This method replaces lesion studies which removed part of the brain, leavingthe animal permanently lacking functions associated with the brain region targeted. Cannulation allowstwo different brain regions to be targeted temporarily and without lasting damage, and enables comparisons to be made within the same animal which is the gold standard for differentiating the functions of distinct brain regions and decreases the overall number of animals needed per study.

Infusion of viral vectors into the brain under anaesthesia to insert designer receptors into localised brain regions and pathways that can subsequently be activated by designer drugs (adult and juvenile).This is the most refined method of brain manipulation currently available and reduces the need to implant cannulae into the brain to target specific brain regions as the designer drugs used to activate the designer receptors can be injected peripherally, thereby minimising stress associated with cannulae implantation and care, and the longer periods of holding time needed for central infusions.
Being held for infusions either into the brain (through implanted cannulae), or peripheral injections (designer drugs) for no more than 5-10 minutes to induce short-acting effects on brain activity and behaviour. Holding the animal avoids the need to use primate chairs which offer no flexibility, unlike ‘holders’ which can adapt to an individual monkey’s needs. The short-acting alterations induced by infusions and injections allow each animal to act as its own control, thereby reducing numbers, and isless invasive and more biologically relevant than inducing permanent changes in brain activity. All animals are habituated to the holding procedure and thus it causes only transient discomfort.

Monitoring of cardiovascular activity remotely in freely moving animals by implanting a telemetry (wireless) probe into the descending aorta in a single surgery under anaesthesia (adult only). Animalsare back in the home cage within 2 hours of having come round from surgery, showing no ill effects. This method allows cardiovascular activity to be measured over the remaining experimental lifetime infreely moving animals thereby removing any

stress induced by restraining the animal to take measurements, and allowing more relevant measurements to be made, e.g., during task performanceand without the stress confounds of being held.

Subcutaneous port implantation alongside jugular vein implantation under anaesthesia to allow subsequent subcutaneous injection of radioactive drugs directly into the jugular (adult only). Animals are back in the home cage within 2 hours of having come round from surgery and show no ill effects. This removes the need for repeatedly performing a painful procedure at the time of the injection, whichmay even have to be performed under sedation.

MR (adult and juvenile) and/or PET (adult only) imaging under anaesthesia for restraint purposes onlyto measure whole brain structural, chemical or functional activity differences. This is the least invasivemethod for measuring whole brain activity.

Surgical procedure whereby a specialised probe is inserted into a localised brain region allowing for the measurement of a range of brain chemicals whilst under anaesthesia (adult). Animals are back in the home cage within 3-4 hours of having come round from surgery with minimal side effects. This ‘invivo’ method avoids the need to sacrifice animals to obtain the same chemical samples.

Challenge with a physiological stressor e.g. cortisol or a pro-inflammatory substance (e.g. BCG) sincesuch stressors are a risk factor for the onset of neuropsychiatric disorders. The dose, route and number of administrations are chosen to minimise suffering.

Injection of non-harmful substances into specific brain regions in a surgical procedure, under anaesthesia in order to determine connectivity patterns in the brain. Animals are back in their homecage within 2 hours coming round from anaesthesia and exhibit no long term negative effects.

Terminal anaesthesia using a sedative followed by sodium pentobarbitone and transcardial perfusion (adult and juvenile). Complete cessation of the heartbeat is confirmed via stethoscope prior to makingincisions for the perfusion for absolute certainty the animal is no longer alive and does not experienceany suffering or distress during the perfusion process.

Overall, we are geared towards optimal refinement, from our choice of animals, to our methods, procedures and skills. Additionally, we make sure that we maintain our high standards and training of staff in order to ensure all our refinements are actually implemented. Thus, I review all procedures and skills of the licenced researchers working in the laboratory, under my supervision, regularly and discussproject licence-related matters at each of my weekly lab meetings.

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

Marmosets are the least sentient organism with a highly evolved prefrontal cortex that controls the expression of higher-order cognitive and emotional behaviour of relevance to our understanding of thecomplex behavioural symptoms of brain disorders.

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

Cortisol levels will be measured in hair and saliva samples taken from live animals via

unregulated procedures, refining where possible the need for more invasive procedures (such as blood sampling).

When developing new techniques, e.g., viral infusion of designer receptors that can be activated exclusively by designer drugs (DREADDS, which activate/inactivate neurons), we collaborate withcolleagues to develop the procedure in rodents, if applicable, before applying it in the marmoset.

Surgical procedures such as collecting chemical samples in a given brain region (called microdialysis)will be performed with non-recovery when appropriate, and the duration of stereotaxic surgeries have been shortened where possible by technical refinements in order to minimise post-surgical complications when recovery is required.

All surgeries, as well as intracranial infusions, are performed aseptically and we have advised numerous national and international labs on best practice to reduce likelihood of post-operativecomplications and associated stress.

Cannulations in marmosets at 12 months of age is at a timepoint when the size of the brain and skull are stable, and thus chronic implants are not likely to cause any more problems than are normally observed for animals above 18 months of age. Initially, we will pilot this in a few marmosets. Prior to cannulation, several adolescent marmosets will be behaviourally screened using the intended tasks toensure that we select the most suitable candidates for cannulation.

As juveniles are still housed with their family group any adaptations required to facilitate these studieswill be discussed in consultation with NACWO and NVS. For instance, we may need to consider how to adapt the diet to motivate testing adolescents without impacting the welfare of the group. We are already in the process of developing a system for assessing the weight and condition of experimental juvenile animals. We will adapt the top of the nest box doors (with flaps and/or rubber) in the home cages of the family pens as we do for our cannulated adult marmosets to avoid the cannulae getting banged accidentally as marmosets pass through the nest box door. In addition, we have performed a limited study using DREADDs to target a specific brain region allowing us to alter activity in that brain region using a systemic drug in the juveniles, reducing the need for cannulae implantation in some cases. This has been successful so will be something we will continue to use wherever possible in thefuture.

This laboratory has been performing many of the described procedures for over 25 years and during this time the techniques have been refined either in house, or in consultation with outside experts in their particular field to minimise pain, suffering, distress or lasting harm. Analgesic, anaesthetic and antibiotic regimes have been developed in consultation with the NVS and are under continual review. We receive additional advice on anaesthesia from an experienced specialist veterinary anaesthetist with considerable expertise in primates. Prophylactic analgesics are routinely administered prior to surgical procedures to minimise pain during the procedure and in recovery. Post-surgical or other procedure-related analgesics and antibiotics (typically oral) are given as recommended by the NVS fora given procedure, but also take into account the condition of the animal, with e.g. some individuals requiring extra pain relief early in recovery and others needing it for longer than the typical recovery window. Animals are closely monitored in the days following surgery, are always provided ad libitum water and normal diet to assist recovery, and monitoring of post-surgical recovery has recently been reviewed in conjunction with the NVS to ensure prompt identification of non-anaesthesia related symptoms, including pain.
A formalised weekly environmental enrichment programme with rotation of enrichment

devices has been instituted in the colony, with new items regularly trialled and added to the rotation if successful. Items such as foraging boxes, highly palatable juice (frozen and liquid forms), swings and baskets, andtextured bedding have all been utilised to enrich accommodation routinely, and/or as part of enhanced recovery after surgical procedures. Live foods (locusts and mealworms) have also been introduced to encourage more natural hunting and foraging behaviours. Animal staff and scientists interact with marmosets on a daily basis, continually monitoring the welfare and environment, ensuring that the NC3Rs guidelines on non-human primate accommodation and care are consistently met and exceeded wherever possible.

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

Our research is constantly guided by, and adheres to the Laboratory Animal Science Association (LASA), the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) and Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines.Not only do we follow the LASA guiding principles of aseptic surgery (http://www.lasa.co.uk/wp- content/uploads/2017/04/ Aseptic-surgery final.pdf), but we have furthered these principles whereverpossible as part of our constant refinement strategy, especially in the case of intra-jugular catheter implantation procedures. We will receive direct updates on best practice from the N3CRs as I have subscribed to their mailing list and we attend the annual Primate Welfare meeting.

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

We have several lines of information that enable us to stay informed about advances in the 3Rs in order to implement them effectively. First, we have registered to the NC3Rs newsletter. Secondly, as allthe project licence holders at our establishment, we receive tremendous support from the staff at the establishment, and we receive regular critical updates from the Named Information and Compliance Officer to which we pay the utmost attention and that we share with all the members of the lab. Third, we regularly hold project licence-related workshops with all the members of the laboratory to discuss the changes in procedures. We also have an excellent working relationship with the animal care staff in our animal facility, which facilitates the implementation of advances in the 3Rs. Finally, we are also partof an international network of marmoset users and we regularly have meetings and exchange best practice.

A retrospective assessment of refinement will be due by 14 October 2028

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 forfuture work of this kind?
- During the project, how did you minimise harm to the animals?

4. Immune Mechanisms and Vaccine Protection

Project duration

5 years 0 months

Project purpose

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

Key words

Vaccine, Pathogen, Lung, Virus

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.

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 aim of the project is to understand how infections causes disease and how vaccines prevent this.

A retrospective assessment of these aims will be due by 18 November 2028

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 we have seen during the COVID-19 pandemic, infectious pathogens can cause considerable global disruption, disease and death. But we also saw how effective vaccines could be at reducing the burden of disease during the pandemic. More and better vaccines are needed to prevent other endemic and pandemic pathogens. In order to do this, we need to understand how pathogens cause disease and how the immune system can be trained to fight them with vaccines. Globally, respiratory infections are the main cause of infectious disease, so we will mainly be looking at vaccines to prevent these, but improved mechanistic understanding about vaccines can be applied across a much broader range of vaccines. One of the challenges is balancing protection and the side effects of vaccines; improved understanding can help with this.

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

We aim to understand the immune response to vaccination and the causes of symptomatic disease following infection, in order to develop better vaccines in the future.

As part of this program of work we will perform:

Pre-clinical validation of vaccine technologies: novel vaccine platforms will be tested to identify approaches to control future pandemics and ongoing endemic infections.

Knowledge generation through the detailed dissection of the immune response to vaccination, this will inform how the immune system senses vaccines and complex interactions between the different cell types that form the immune response leading to protection. This will inform vaccine design for a wide range of diseases.

Knowledge generation of the response to infection. Dissecting disease following infection, will potentially identify targets for future interventions.

Outputs of this work will be disseminated to the scientific community through publication in peer reviewed journals and presentation at scientific conferences.

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

Knowledge generation: the data from this project will contribute to the scientific understanding of vaccines and infections. This knowledge will benefit other scientific researchers, vaccine developers and healthcare professionals.

In the longer term, the knowledge generated will contribute to a broader understanding of the immune response to infection. These questions are important to other immunologists, vaccine developers and potentially also the development of protein replacement through RNA delivery.

Product development and validation: Working in partnership with biotech and pharmaceutical companies, we will explore vaccine mediated protection; this will lead to the development of new vaccines. For example, we have worked with major vaccine developers in the last project, leading to successful vaccine deployment. The development of new vaccines will be of benefit to everyone as they are a population medicine.

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

1. Collaboration with academic and industrial partners.

2. Dissemination of knowledge through standard scientific routes (papers/ meetings), social media (blogs/ twitter) and scientific engagement (festivals, books). The PI is also an experienced science communicator, speaking to journalists in written media, radio and TV.

3. Where appropriate, unsuccessful approaches will be published as part of larger data sets.

Species and numbers of animals expected to be used

- Mice: 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 are only using mice in this study. Mice recapitulate many aspects of the human immune response. Mice and humans have about 30,000 genes of which 1% are species-specific. Equivalent mouse genes have been found for all genes known to cause human disease, and 99% of mouse genes have a human homologue. Although our immune systems have diverged during the 75 million years since separation, the same immunological niche is sometimes occupied by different proteins because of convergent functional development.

There are many benefits to the mouse model: the mouse is the only species for which many of the research tools are available to study mechanism. The mouse genome is one of the best characterised, with many knockout strains available to investigate important research questions. Other species may be moderately more reflective of some aspects of the human immune response, but the interventional mechanistic studies proposed are simply not possible in them. All studies will be informed by in vitro work to determine correct doses of virus and drugs.

We will also in some studies be using neonatal and juvenile mice. In general, respiratory infections are more severe in early life; likewise vaccines are often less protective. Some of this is driven by an immature immune system. Performing comparisons between early and later life is important to understand these differences.

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

We are interested in the outcome of infection, predominantly respiratory infection (e.g. Influenza) and how vaccines, drugs or treatments affect that.

Most animals will receive some form of infection. This will normally be preceded by a vaccine or drug administration. Monitoring in the blood may occur during the study. Most animals will undergo a single infection.

Standard experiment:

Animals will undergo the following typical procedures:

A. For the testing of a vaccine:

1) Vaccination – normally 2 doses, this normally has no adverse effects, but some vaccines can have a brief acute systemic effect. Vaccines are most commonly delivered by the intramuscular route.

2) Blood sampling by the tail vein to assess immune response to vaccine – this is normally done twice per study.

3) Pathogen inoculation via intranasal route, under general anaesthetic. Normally a single infection, though may be up to 3.

4) Monitoring extent of vaccine protection against infection through signs of disease in infected animals.

5) Mice will be humanely killed. A normal vaccine study takes 8 weeks, to allow the immune response to develop.

B. To investigate disease after infection

1) Use of drugs to manipulate disease severity; these drugs will either be administered intranasally (the site of infection) or systemically by injection.

2) Pathogen inoculation via intranasal inoculation, under general anaesthetic; normally a single infection, but occasionally we will assess the affect of one infection upon another.

3) Monitoring protection infection severity through signs of disease in infected animals.

4) Mice will be humanely killed. Infection studies normally take one week which the peak of symptoms.

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

The procedure that is most likely to cause adverse effects in the animals is infection.

Infections will occasionally result in some transient discomfort for the animals. This is most likely to be seen in control-unimmunised or untreated mice. But the use of infection is central to these studies; it is the best and most stringent way to identify the protection mediated by the vaccine platforms.

Most infections will lead to transient adverse effects. These include clinical signs such as lethargy, faster breathing and a small amount of weight loss.

Occasionally, mice will experience more severe, transient responses to infection, including substantial loss of body weight (up to 25%) and sustained signs of ill health (such as greatly reduced activity, rapid breathing) over a period of several days. The duration depends on the infecting pathogen, but there is normally a 3 day period over which where the adverse effects peak before recovery or humane killing.

Some other procedures – for example drug administration or vaccination can cause a low level, short term weight loss and reduced activity for less 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)?

Mild (studies without infection) 20%

Moderate (infection studies) 70%

Severe (minority of infection studies) 10%

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 November 2028

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 is based on the study of the interactions of vaccine and host in the context of an intact immune system. Investigating individual cells is informative and in vitro studies are being performed in parallel. But the immune response comprises multiple different cell types working in concert and some of the analysis of this multi-component system can only be performed in vivo using animal models.

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

We screen vaccines in vitro, for example with RNA vaccines we measure whether there are differences in the amount of protein made when cells are treated with the vaccine: this is important because the more protein, the better the immune response.

Why were they not suitable?

In vitro cell models can only inform us about how individual cells respond to the vaccines, but not about the complex interplay across multiple cell types. One question of particular importance is the kinetic of the response between injection site, lymph node (where the immune response is initiated) and the potential infection site and how these work together. In vitro models cannot give us this information.

A retrospective assessment of replacement will be due by 18 November 2028

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 draws on previous experience/ earlier project licenses and the volume of work required to confidently address the scientific questions.

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

The studies are carefully planned to reduce animal numbers, overlapping control groups are used where possible.

We will only perform studies on animals when there is no other alternative. We will reduce the numbers of animals used by extensively testing our hypothesis in experiments without animals before confirmation studies in animals.

When it comes to the use of animals, we will use statistical advice and our longstanding experience to minimise the number of animals needed to answer each research question. Experimental design is informed by 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 will start all our experiments with a small pilot of a few animals to be sure that there will be no unexpected welfare harms and to see if it is needed to go on to a large experiment with more animals. We will collect as much information as possible from every animal for example, making many measurements from the same animal over time. We will also collect tissues from all our animals, and share with other researchers, to perform experiments in the laboratory so no additional animals are required.

The use of sufficient pathogen to result in transient weight loss provides a method for non-invasive monitoring of disease meaning effects of treatment can be rapidly evaluated without the use of larger numbers of animals.

A retrospective assessment of reduction will be due by 18 November 2028

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 this study for several reasons, there is a large number of tools available to interrogate the immune response, they are widely used so responses are comparable between different studies, they are the lowest mammal in which these studies can be performed and they recapitulate most features of the human immune response.

The most severe procedure in the studies is the use of infection (e.g. influenza, RSV, antimicrobial resistant bacteria). The central goal of this project is to understanding what causes disease after infection in order to reduce disease severity through vaccination or drug treatment. We are mainly using respiratory infection models (both viral and bacterial). We have 20 years' experience of these models, and have refined them throughout this time. We use the lowest dose to cause symptomatic disease, where new batches of pathogen are used we perform small pilot studies to ensure the dose causes the minimum harm. Animals are closely monitored to ensure harms are minimal. Additional support, for example food on floor of cages, extra bedding and occasionally wet mash are used.

Studies are terminated in accordance with severity and scientific endpoints, to ensure each study generates high quality data that will address the aims of the project. Most infection studies are terminal, or lower doses are used where recovery is needed to look at long term immune memory.

Other procedures are mild, or cause transient low level distress in the animals. These all draw on previous experience and are refined to cause the minimum suffering, for example through anaesthesia where pain might be local and transient. Models of vaccination include intramuscular delivery of experimental vaccines for example RNA.

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

The immune response to vaccination and infection is a complex, multi-stage response over time. We need to investigate changes over time. So terminal anaesthesia is not possible.

Lower stage animals do not have an adaptive immune response that reflects the human situation.

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

We will use situation specific monitoring, with increased intensity at known times of severity or for new/ pilot studies. Where problems arise, we shall consult the NACWO and veterinary surgeon and offer pain relief, treatment or humanely kill 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?

Through 3Rs seminars at workplace, and contact with 3Rs lead. Through reading current literature and discussion at conferences.

A retrospective assessment of refinement will be due by 18 November 2028

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?

5. Interventions against Tuberculosis

Project duration

5 years 0 months

Project purpose

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

Key words

Vaccine, Therapeutic, Efficacy, Tuberculosis, Immunogenicity

Animal types	Life stages
Rhesus macaques	adult, neonate, juvenile
Cynomolgus macaques	adult, 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.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Uses non-human primates

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 assess new vaccines, therapies and treatments in refined characterised non-human primate models of Mycobacterium tuberculosis infection

A retrospective assessment of these aims will be due by 18 November 2028

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?

Despite a widely used vaccine being readily available, tuberculosis has been the leading cause of death of humans from a single infectious agent worldwide, responsible for 1.5 million deaths in 2020 alone. An estimated 10 million people fell ill with TB in 2020, including 1.1 million children (WHO, 2021). The SARS-CoV-2 pandemic has likely reversed several years of global progress in reducing TB mortality with projections suggesting that the number of deaths could continue to increase over the years to follow. New vaccines and drugs to combat tuberculosis are urgently needed.

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

The programme of work described in this application responds to the international need for NHP models with which to assess new TB interventions and will provide

- Well characterised models of the human respiratory pathogen tuberculosis to allow the evaluation of new vaccines and therapeutics.
- Evidence of vaccine and therapeutic efficacy.
- Publications in peer reviewed journals and presentations at expert meetings and conferences that will advance the field.
- Refinement of the models applied.

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

Data obtained from studies will the assist progression of new TB vaccines and drugs through the developmental pipeline.

Knowledge gained will be transferred to subsequent studies in an iterative process. New interventions against tuberculosis will benefit human health

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

The work is highly collaborative with assessments of new vaccines and therapeutics conducted as collaborative efforts with academia, national and international public health bodies and project consortia.

Knowledge and findings will be transferred to subsequent studies and to influence the clinical arena through design of clinical trials.

Disseminated through the scientific community through presentations at meetings, publication.

Species and numbers of animals expected to be used

- Rhesus macaques: 400
- Cynomolgus macaques: 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.

Non-human primate (NHP) models provide the most relevant pre-clinical models of human tuberculosis because of the similarities between the macaque and human immune systems and the response to TB infection. Adult animals are most suitable for these studies.

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

Wherever appropriate for the type of procedure animals will be sedated. These may include collection of body weight and temperature, examination for gross abnormalities, vaccination, collection of small blood samples, bronchiolar lavage (BAL), CT or Pet-CT scan, pathogen challenge, or euthanasia.

Typical study durations are 20 weeks for assessment of immunogenicity, twelve to sixteen weeks for assessment of the outcome of mycobacterial challenge and eight weeks to determine the impact of drug treatment. Typically, clinical examinations and blood sample collections are performed at two to four weekly intervals, BAL are collected at least four weeks apart on one or two occasions during immunogenicity studies and scanning is conducted at monthly intervals after mycobacterial exposure. Experience shows that no adverse effects are expected and the level of severity will be mild. However, animals will be monitored after sampling to ensure that there are no adverse effects. At the end of study, tissue samples will be taken from macaques which have been terminally anaesthetised and then exsanguinated.

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

Transient stress due to induction of sedation/anaesthesia may occur during recovery or non-recovery procedures. This will be minimised by use of rapid-acting sedatives or anaesthetics appropriate for the procedure and species based on experience and veterinary advice and by providing a suitable protective environment and level of observation until fully recovered. Experience with the macaques used under previous licences has shown that there are few effects from the sedation and blood sampling regimes to be used. The frequency and volume of blood samples will be kept to the minimum required to assess immune and clinical status and in line with the Standard guidelines for withdrawal of blood as outlined in Wolfensohn and Lloyd (2013). Red cell haemoglobin concentration may decrease and if at any stage in the study a concentration significantly less than normal for the individual is detected the blood volume collected will be reduced. All animals will be closely observed after all procedures and advice sought from the NVS regarding any adverse effects.

Any vaccines given will normally be formulated for human use and will consequently utilise adjuvants of known safety. Previous experience with BCG and vector-based vaccines in macaques has shown minimal side effects. These include transient enlargement of lymph nodes and some swelling or a small lesion at the site of immunisation. BCG has a well-documented safety record and is used to immunise human infants in TB endemic areas. Where any novel adjuvants or therapeutics are used, or where the route of administration is novel, there will normally be supporting data from other animal species.

Where bronchoscopy is used previous experience indicates that bronchioalveolar washes performed using multiple aliquots of no more than 20ml of physiologically compatible fluid can be administered and recovered without adverse effects. Only staff trained in these procedures will be used.

Protocols and procedures including anaesthetic regimes were established and refined under previous licences. During scan collection animals will be maintained by experienced staff following veterinary guidance monitored for clinical well-being using parameters such as pulse oximetry and capnography.

Contrast media and radiotracers will be those in common use in both veterinary and medical practice and adverse effects are not expected. Injection of contrast media can induce nausea in a small proportion of rhesus macaques however the risk of this is significantly reduced by intravenous injection of the anti-nausea drug Maropitant prior to scanning. Adverse effects from jet ventilation are not expected. Carbon Dioxide levels will be monitored during the process to ensure levels remain within the expected range and that ventilation is appropriate.

Following infection with Mycobacterium tuberculosis animals may experience weight loss, a transient increase in body temperature, and pulmonary changes. Animals will be closely monitored for behavioural and clinical changes which will inform on progression to 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)?

Robust humane end points, based on changes in the clinical parameters, have been established for both rhesus and cynomolgus macaques under previous licences

The expected severity resulting from the assessment of immunogenicity is mild.

The expected severity in studies to evaluate the efficacy of vaccines and therapeutics following mycobacterial challenge will be moderate at most, depending on the effectiveness of the intervention. It is estimated that approximately 25% - 33% of untreated animals are likely to experience moderate levels of severity and the remaining 66% - 75% of untreated animals are likely to experience mild severity. As only the most promising vaccines and drugs which have strong supporting evidence from assessments in other systems will be considered for testing in NHP models, it is anticipated that > 75% of treated animals are likely to experience 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 18 November 2028

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 complex interactions between pathogens such as Mycobacterium tuberculosis and the host are difficult to model without the use of a complete living animal where the target cells, the innate defence mechanisms of the respiratory tract and lung and the innate and acquired responses of the immune system are all fully functional as they would be in man. Similarly at this stage in the testing of a vaccine or therapeutic candidate, the complexity of immune responses and the effects of infection on specific cell populations, the dynamics of the inter-relationship between cell populations and host and the development of immunity cannot be reproduced in a cell or tissue culture environment. It is necessary to reproduce as closely as possible this complex system in an experimental situation. Currently the only way to reflect this complexity and to define the dynamics of what is occurring in real time, is to use animal models that are reproducible and have been demonstrated to reflect the changes seen in man.

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

Computer modelling and in vitro systems.

Why were they not suitable?

Whilst every effort will be made to use computer modelling and in vitro systems to model aerosol survival and transmission, vaccine development and the screening of potential therapeutics, a realistic animal model is required to provide robust data on efficacy and immunogenicity. In vitro systems can provide useful information on key interactions between system components, but they lack the complexity required to reflect all the interactions between host and pathogen that may influence the outcome of vaccination or treatment. We actively seek and work with collaborators developing in vitro alternatives and materials collected from studies performed under the previous licences has been shared to support assay development and this will continue as new initiatives come along. Computer modelling has the capacity to model complex environments, but models remain a simulation dependent on the information available which limits their predictive power as there is still much to understand about the interactions within the multifaceted host immune system. Only in an animal model can we currently demonstrate efficacy in terms of alleviation of clinical signs and systemic pathology.

A retrospective assessment of replacement will be due by 18 November 2028

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?

Numbers were estimated based on contracted and potential future study requirements informed by experience gained under previous licence periods. The study designs and experimental group sizes that will be used have been discussed with our consultant statistician. The statistical power of the challenge studies has been determined to use the minimum numbers of animals and studies conducted under previous licences have shown these to be sufficient to identify significant treatment effects

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

The project team has a wealth of experience with the models built up over more than twenty years. The study designs and experimental group sizes to be used have been discussed with our consultant statistician. Power calculations were performed to determine appropriate group sizes for efficacy studies using the aerosol challenge models developed under the previous licences based on pathology score data obtained from experiments conducted to date. All opportunities to inform and optimise new study designs will be taken including, knowledge from previous studies, information from published reports, discussions with experts in the field and the use of tools such as the NC3R's Experimental Design Assistant. Longitudinal sampling of each individual over a number of time points is included in study designs and will increase the power of studies. The collection of samples and data from individuals before and after vaccination or infectious challenge, means that treatment effects can be determine within individuals through comparison of measurements collected before and after such events thus removing the need for additional untreated control groups.

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

All measures possible will be taken to control variability to allow for smaller group sizes and reduce the risk of the need to repeat studies. The techniques for sample collection and challenge used with non- human primate models are well established and all staff involved are trained and highly experienced.

Intrinsic inter-sample variation will be kept to a minimum as animals are maintained in consistent controlled environments and sample collections are performed at the same time of day on each and where possible with standard operators. This ensures the reproducibility of the infection, clinical monitoring, sample collection and analysis procedures which allows animal numbers to be kept to a minimum. To maximise the information from each animal, all relevant materials, as practicable, are stored to provide an archive that can be evaluated using new techniques they become available which reduces the need to repeat in vivo studies. The culture stocks of the pathogens used for in vivo challenges are well characterised, and all information available from previous in vitro and in vivo studies will be used to support new studies to ensure the animal numbers are kept to a minimum. For studies using stocks that have been previously characterised in non-human primate studies there is a wealth of data which will allow numbers, particularly in control groups, to be reduced. Future work will continue to use animals from the same source as those used in previous studies. This use of animals of the same genetic background will ensure consistency in established models. Studies will be designed to ensure appropriate consistency in animal gender and age range. Previous studies compared the rhesus and cynomolgus macaques from these breeding colonies in terms of their response to vaccination with BCG and infection with Mycobacterium tuberculosis. This knowledge will assist selection of animals with the most appropriate characteristics for each study hence minimising the number required.

Wherever possible potential vaccines and therapeutic compounds will be pre-screened both in vitro and in smaller animal species such as mice and guinea pigs to ensure only the most effective interventions are tested in this model thereby minimising the number of animals used. The use of 'in life' imaging during studies provides the opportunity to significantly reduce the number of animals required for studies as it permits the evaluation of disease in the same animal on multiple occasions. Thus, imaging enables the sequential evaluation of disease needed to monitor the evolution of disease or treatment effect, as well as the quantification of disease burden without the need for the serial killing of groups.

Pilot studies may be performed to evaluate new parameters such as, mycobacterial challenge strains, or the utility of new models and will inform follow on studies.

A retrospective assessment of reduction will be due by 18 November 2028

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 for these studies will be housed in socially compatible groups with various enrichment strategies available throughout these experiments. Animals will be vaccinated and / or challenged with infectious respiratory pathogens and blood samples and information on clinical parameters collected at fortnightly to monthly intervals. Wherever appropriate for the type of procedure, animals will be sedated to minimise stress. Wherever possible pre-challenge samples will be taken, and vaccinations given whilst the animals are still within the colony environment. Considerable experience has now been gained in immunisation and aerosol challenge of macaques which has provided a wealth of robust clinical, behavioural and immunological data that can be used to identify individuals that will develop progressive disease and allows early intervention using humane endpoints.

Access to clinical trial data of similarly vaccinated human volunteers and the use of identical immunological assays indicates that the macaque model provides the best opportunity to calibrate pre- clinical responses with those seen in humans. Understanding the relationship between outcome of vaccination in humans and macaque species will allow selection of the most appropriate species for studies.

The established expertise in the use of medical imaging to visualise and quantify TB-induced disease in-life not only provides information that informs the clinical management of animals during study but also increases the scientific knowledge gained from each study. The ability to quantify subtle pathological changes through imaging provides

opportunity to reduce the need to progress to higher levels of disease and thus to reduce the severity experienced during a study.

The image collection process has been further refined through the introduction of jet ventilation using a positive end-expiratory pressure (PEEP) device, in combination with injectable anaesthesia, to minimise respiratory motion with consequent improvements to welfare during scan collection

The creation of the in vivo imaging capability was critical to the development of the low dose aerosol challenge models established under the current licence. These refined models use lower challenge doses more akin to that seen in natural human infection, which rarely induce progressive disease and more refined, quantifiable early end points that do not depend on the development of progressive high burden disease.

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

Studies in macaque models will only be considered to address clinically relevant questions with only the most promising new interventions. A stepwise approach is taken to the development of new TB interventions. The new candidates that progress to in vivo testing are initially evaluated in small animal models. Mouse models are generally used as a first screen of vaccine candidates and are very useful for studying detailed immunological responses. Guinea pigs are considered a more stringent model than mice to discriminate between vaccines in terms of protective efficacy, since they show a variety of pulmonary and extra-pulmonary lesion types that are similar to those observed in humans. Macaques provide the best model for the evaluation of vaccine or therapeutic efficacy as their physiology and immunology is very similar to man. This similarity allows the application of assays identical to those used in human clinical trials. NHP are reserved for end-stage testing of only the best new candidates prior to their large-scale evaluation in humans.

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

Studies will use the smallest number of animals and procedures to minimise the welfare costs.

To avoid any undue stress, animals will be sedated before blood is collected from a superficial vessel with the use of rapid-acting sedatives/anaesthetics based on current veterinary advice. All animals will be placed in a suitable protective environment and monitored until fully recovered

Throughout the studies animals will be rigorous assessed with monitoring frequency dependent on the severity of clinical signs presented and increased accordingly.

Studies include the collection of thoracic and abdominal images which provide detailed information on clinical status. A comprehensive and wide-ranging assessment is made of the condition of the animal throughout the study period and decisions taken based previous experience with the model.

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

The following published guidelines will be followed where appropriate

- Local AWERB guidelines.

- Guidance on Animal Testing and Research from the Home Office.
- The Norecopa PREPARE guidelines.
- Good research practice guidelines from the Wellcome Trust.
- LASA and RSPCA guidelines.
- The Handbook of Primate Husbandry and Welfare by Sarah Wolfensohn and Paul Honess.

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

Information on the 3Rs will be obtained by attendance at meetings such as the NC3R's primate welfare meeting, conferences, continued discussion with peers, and the use of on-line tools such as the dedicated NC3R's macaque website and the monthly NC3R's News bulletin.

The institute has robust channels in place for the dissemination of information related to the 3R's e.g., via the Animal Welfare Review Body.

A retrospective assessment of refinement will be due by 18 November 2028

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?

6. The Function of Key Proteins in T Cell Signalling and Disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Cancer, immunotherapy, viral infection, immune cells

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.

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 main objective of this application is to study the function of glycogen synthase kinase 3 in the immune system. We have previously been shown that treating mice with drugs that act against GSK- 3 can suppress tumour growth or viral spread. However the mechanism behind this is unknown, during this project we plan to investigate this further and uncover how GSK-3 modulates tumour growth and viral spread.

A retrospective assessment of these aims will be due by 21 October 2028

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 primary benefit of this research would be to obtain a more complete understanding how GSK-3 functions in the immune response. This could further knowledge in the scientific field and result in the design of new or improved treatments for cancer and viral infections. Many available drugs are not target-specific and can suppress the whole immune system leading to other infections which can be fatal, improving specificity or identifying new proteins which can be targeted specifically is of extreme importance.

There are 6 protocols in this project:

1- Breeding -To produce, maintain and provide genetically altered mice.

2- Vaccination - To investigate the immune response to antigens, antibodies, anti-cancer drugs and/or enzyme inhibitors

3- Cell transplantation - To investigate immune cell differentiation, migration or apoptosis.

4- Tumour cell growth - To investigate the effect of substances (i.e antigens or antibodies) and/or cells on tumour development/growth.

5- Viral infection - To investigate the effect of substances (i.e antigens or antibodies) and/or cells on viral spread.

6- Immune modulation of tumour cell growth - To investigate the effect of oncolytic virus in combination with substances (i.e antibodies or inhibitors) on tumour development/growth.

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

This project aims to uncover the mechanisms behind the regulatory role of GSK-3 in the immune response broadening scientific knowledge and providing insight into improving current therapeutic approaches. This will ultimately lead to high impact publications as well as novel therapeutic applications and drug development. Previous work has already led to preclinical studies in which the drug utilised is now in clinical trials.

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

Academic Research: The findings of this research proposal are likely to be of interest to scientists across various different disciplines, both in basic and applied research fields, within the UK and abroad. In addition to cancer biologists, academics in basic research fields studying checkpoint blockade are likely to benefit from the results of this research.

Industry: The findings from this will benefit the pharmaceutical industry and could lead to the possibility of other small molecule inhibitors being introduced as alternative therapies which will benefit patients. My studies directly relate to immune checkpoint blockade, which is an ever-growing area of therapeutic potential with much success clinically. Enhancing current therapies or providing alternatives remains an important target for UK

based biosciences industry, as modulators and drugs targeting this process are valuable commercial assets.

Healthcare: This work may lead to the development of new small molecule drugs which can be used in the treatment of cancer and/or viral infections. My work so far has already compared GSK-3 inhibitors with anti-PD1 antibody therapy (in murine models) showing similar effects. If successful translationally, with the current small molecule compounds or ones designed against newly identified targets - as an outcome of this project, these could represent cost-effective methods, which will ultimately increase healthcare efficiency and decrease the burden on the National Health Service.

Ultimately, if this study contributes towards the development of novel small molecule compounds that can regulate tumour growth, the general public will be the main beneficiary in the long run. Also, as mentioned above, knowledge generated from this work may also lead to devising novel treatments for viral infections, ultimately benefiting public health.

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

Materials made in these studies (i.e. mouse strains, antibodies, cell lines, DNA constructs, etc) will be made freely available to the scientific community, while information gained from these studies will be published in peer-reviewed journals.

Species and numbers of animals expected to be used

- Mice: 20200

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 plan to use mouse tumour models for our experiments, which constitute the lowest form of mammal recognised as being relevant to human cancers. To achieve the aims of the project, we will use mice at the adult stage of life to ensure we can study a fully-functioning immune system to provide meaningful data towards clinical trial applications in humans.

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

The vast majority of animals will be injected with tumour cells which will be allowed to grow and form established tumours. These cells may be administered surgically for intracranial implantation or through injection for extracranial tumour growth. Animals will receive treatment with various drugs, including immune-stimulating drugs and enzyme inhibitors. This treatment will be given through the least intrusive route possible, most likely intraperitoneal injection although this will be determined for each drug. Tumours will be measured and examined in order to assess the effects of therapy. Typical experiments using B16 melanoma will have a duration of approximately 1 month starting with the initial tumour cell implantation and treatment injections every 48 hrs over a 3 week period. During this time, in vivo imaging will be performed under anaesthetic to visualise tumour growth. Multiple treatments and/or imaging will be combined where possible to minimise

the number of procedures. Animals responding to treatment may be kept alive to a maximum of 15 months to demonstrate longevity of any effects seen.

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

Procedures involving treatment with various drugs or radiotherapy may cause transient discomfort to the mice, the majority of which will be from the administration of the treatment e.g. needlestick.

Extracranial tumour growth:

Injections of tumour cells and the resulting growth of cancer masses (to a maximum of 15mm in diameter) may cause some transient discomfort but no material effect on the well-being of animals.

Intracranial tumour growth:

Mice subjected to intracranial injection of tumour cells will experience temporal discomfort from the surgery and anaesthesia. The injected tumour cell volume is small (2-3 uL) and only temporarily affects the animals

In survival experiments individual mice are culled when they display moderate signs. However, uncommonly, intracranial haemorrhage may occur at the tumour site resulting in sudden death without any prior clinical signs (<5% of animals undergoing intracranial tumour cell implantation). In the previous licence, we observed sudden deaths in 7 out of 500 mice (~1.4%) when using an increased (3 times daily) monitoring protocol. In the case of intracranial tumour studies, this increased monitoring of 3 times daily will be adhered to.

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 (>90%) on breeding protocol 1 are not expected to exceed mild adverse effects

The majority (>90%) of animals on experimental protocols 2- 6 are not expected to suffer more than a moderate degree of adverse effects during the entirety of our experiments. However, occasionally at the endpoint a small number (<5%) of mice may display severe symptoms, due to possible higher rate of tumour haemorrhage with the GA Mice. In the previous licence, we observed sudden deaths in 7 out of 500 mice (~1.4%) when using an increased (3 times daily) monitoring protocol.

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

- Killed
- Kept alive
- Used in other projects

A retrospective assessment of these predicted harms will be due by 21 October 2028

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?

Mice need to be used to assess the function of these adaptors in in vivo function. The need for animal models has arisen from extensive previous studies with in vitro experiments where the use of in vitro cell lines produced inconsistent results that could only be resolved or confirmed with the use of an animal model.

Further to this, in vitro work has given rise to possible candidate genes as potential anti-cancer/viral drug targets and it is essential to validating these genes in a fully-functioning competent immune system and to analyse their function in tumour/viral development.

Which non-animal alternatives did you consider for use in this project? Laboratory studies using patient-derived samples including blood and tumours. Why were they not suitable? Immune responses in patients involve complex interactions of multiple cell types in different sites within the body and these interactions cannot be fully replicated in a laboratory setting without the use of animals.

A retrospective assessment of replacement will be due by 21 October 2028

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?

Statistical analysis will be used to determine the minimum numbers of mice used, while ensuring sufficient data are generated to produce meaningful results. The use of pilot studies (these will be small groups of 3 mice per treatment group) will help to assess animal numbers and how best to design the main study in order to gain maximum information.

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

When designing experiments, statistical analysis, including power calculations, will be performed to ensure that we use the minimum number of animals per group to be informative and to provide robust results.

Our experiments will be conducted using genetically identical animals that are free of obvious disease; this avoids the variability of treatment response associated with non-identical animals and thus

reduces the required sample size.

Initially, preliminary non-animal laboratory work will be used to guide therapeutic dosing and treatment schedules, which will be evaluated in pilot studies involving small groups of animals. These short-term studies will guide the most appropriate doses, schedules and combinations of therapies to be undertaken in longer-term experiments. Group sizes for individual experiments will be based on efficacy seen in these preliminary experiments. For new models, study design will be discussed with statisticians prior to planning large experiments

Non-invasive techniques such as MRI and Luciferase imaging have also played an important role in experimental design. The latter can be used to label cells in adoptive transfer experiments to detect homing of cells and migration. It has also been used for both viral and tumour protocols where the virus/tumour cells used to infect the animals is luciferase tagged. For both techniques the whole mouse can be imaged on multiple occasions to track the spread of disease or cell movement without the need of culling the animal. Previously mice had to be culled at the different time points and this involved much higher numbers of animals being utilised.

We have utilised the NC3R's Experimental Design Assistant to plan experiments and sample size has been determined using Power calculations as determined using G*power 3.0.10.

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 collect tissue 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.

For instance, in protocol 4, mice will where possible be injected with tumour cells in two sites – one site for control tumour cells (e.e. wildtype tumour cell line) and the other site for modified tumour cells (e.g ova peptide expressing tumour cells). This allows a control tumour to develop in the same animal as the test tumour and hence eliminating the need for subsequent control.

Non-invasive imaging techniques in live animals and analysis of tissue samples collected after sacrifice of the animal will allow us to maximise data collection during and after experiments and reduce the total number of animals required.

A retrospective assessment of reduction will be due by 21 October 2028

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 welfarecosts (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 our experiments will be conducted in mice. Many of the experiments planned will utilise a superficial tumour model, which is a short-term model that does not require the added stress of anaesthesia for tumour measurements. The severity of the procedures used will be kept to a minimum, by combining procedures where possible, using the least intrusive techniques and ensuring appropriate humane endpoints. The majority of the studies detailed in this project will not exceed a ‘moderate’ degree of animal suffering as useful data can be obtained from animals bearing low tumour burden, with the animals being killed after only short periods of tumour growth.

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

The use of less sentient animals or those terminally anaesthetised is not possible here due to the need for the whole animal physiologically to enable tumour growth. Mice constitute the lowest mammal recognised to be relevant for human cancers and the aim of this project is to provide meaningful data for progression of these approaches/treatments to patient care.

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

Animals will be monitored daily for any signs of distress that might affect their well-being. Such signs of distress include loss of appetite, huddling, shivering or any abnormal behavioural problem. Any mice that show persisting signs of distress (over 24hr period – see scoring system below)) will be humanely killed.

Superficial tumours may develop signs of ulceration, irritation or reddening. Animals developing such symptoms will be closely monitored and where needed pain controlled through use of analgesia.

Tumours will be monitored using a tumour scoring system (shown below) and humane endpoints will be based on a both tumour size as well as tumour ulceration in combination with general well-being of the animal.

Risk of infections are minimised ensuring all surgeries are performed aseptically under general anaesthesia and a separate new needle is used for each injection performed. The latter is also important for minimising harm to the animals.

Analgesics are administered to minimize pain.

Intracranial injection of cancer cells may result in brain haemorrhage. During the previous project, we amended the protocol several times to increase the general monitoring of animals for any signs of distress and to monitor tumour size by bioluminescence imaging which enables us to terminate experiments prior to occurrence of neurological symptoms caused by tumour growth in the brain.

Subcutaneous tumours are removed, or the animals are killed before the tumour exceeds diameter of 1.5 cm. At this size the tumours have minimal effect on the animals.

The severity of the procedures used will be kept to a minimum, whilst providing meaningful data for translation of these approaches to patient care. Discomfort and distress experienced by the animals will be limited to unavoidable procedures required for the conduct of sound research.

All methods will follow LASA guiding principles for preparing for and undertaking aseptic surgery; LASA Good practice guidelines on administration of substances; NC3Rs blood sampling guidelines.

For delivery of therapeutic agents, daily maximum volume and number of injections will not exceed the limits recommended in the guidelines above.

Animals will be examined daily and adverse effects will be scored according to the following criteria.

Score:

0 – Natural activity, behaviour and appearance

1 – For each of the following: Weight loss (up to 5% body mass), lesion/ulceration with dry scab OR abnormal gait not impeding locomotion

2 – For each of the following: Weight loss (up to 10%), Ulceration/lesion 2-3mm growing OR weeping

3 – Any of the following: hunched position, isolation, piloerection, inactivity, weight loss up to 15%, partial paralysis, tumour diameter of 15mm or Ulceration/lesion 3- 5mm growing OR bleeding.

Action to be taken based on total score from above:

0 – Normal, no action required

1 – Development of clinical symptoms. Frequency of observation should be increased to twice daily.

2- If symptoms worsen or persist for 24 hrs, animal should be humanely killed without delay.

3 –Animal will be humanely killed.

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

All methods will follow LASA guiding principles for preparing for and undertaking aseptic surgery; LASA Good practice guidelines on administration of substances; NC3Rs blood sampling guidelines.

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 advances in the 3Rs by keeping up to date with correspondence regarding this and ensuring attendance of the PPL holder and any PIL holders working on this licence at the relevant meetings.

A retrospective assessment of refinement will be due by 21 October 2028

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?

7. Understanding the Cell Biology behind Cardiovascular Diseases

Project duration

5 years 0 months

Project purpose

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

Key words

cardiovascular disease, immune system, cell proliferation, cell death, cell senescence

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 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?

To understand the interplay between fundamental processes of cell biology (cell proliferation, death and ageing) and the immune system in cardiovascular diseases, such as atherosclerosis, stroke, heart failure and aneurysm. In particular, these processes will be studied in the arteries and heart.

A retrospective assessment of these aims will be due by 09 November 2028

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?

Cardiovascular diseases (CVDs) are responsible for 40% of all deaths by cause, representing 18 million deaths globally each year. However, although many more people now survive, e.g., heart attacks and stroke, the debilitating consequences on quality of life and high risk of a secondary event leads to a huge economic and social burden.

Thus, unravelling the underlying biology that both causes CVD and dictates the outcome after a CV event is vital to both prevent disease occurring and to mitigate the subsequent clinical consequences.

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

The proposed research will reveal how basic cell biological processes such as proliferation, death and ageing affects the immune system and how this leads to changes in the arteries and heart that drives disease. The results should lead to better therapeutic targets that could one day prevent heart attacks, strokes and aneurysm, and/or limit the consequences after having one.

Our results will be published in peer-reviewed scientific journals, presented at scientific conferences and where appropriate would lead to patent applications.

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

Cardiovascular disease (CVD) accounts for 40% of all deaths by cause, and those surviving have long- term impact on their health and well being, along with huge socio-economic burden to the economy (e.g. CVD costs the UK ~£20 billion/year). Thus, the rigorous scientific study of CVD is of the utmost importance.

In the short term the main benefactors of this research are the scientific community through the development of new knowledge on the disease process, along with new models to better study aspects of CVD and cell biology.

In the long term the main benefactors of this research would be patients, via the identification of new biological processes that could be therapeutically targeted without side effects such as infection

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

We collaborate with a large group of scientists and clinicians to address scientific problems from multiple angles. We actively present work in progress locally, nationally and internationally at seminars and meetings to maximise information exchange and increase the chance of fruitful collaborations. I am a member of several learned scientific societies, specifically created for themed knowledge dissemination.

In addition, we will publish our results in suitable journals and/or pre-print servers, regardless of whether the scientific outcome was as expected (i.e. the experiment 'worked'), or otherwise, along with discussion at scientific meetings.

Species and numbers of animals expected to be used

- Mice: 16,050

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 utilise mice to study CVD, as they represent the 'lowest' animal that can be genetically manipulated, has scientific reagents available to study them and, most importantly, generate human- like disease in a time frame that can be usefully studied (i.e. ~3 months). We are using adult mice as non-inherited human CVD disease develops in adults, not children.

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

Typically, animals will be treated with a drug (e.g. injections); or have its immune cells manipulated (e.g. a bone marrow transplant); or will be genetically altered (knock-out of genes) in a way suspected to alter disease. Disease will then be induced by, e.g., high fat feeding for 12w (to induce atherosclerosis) or surgical manipulation (e.g. to induce a heart attack). Finally, disease progression and outcome will be measured in several ways to assess the impact of the treatment, both while the mice are still alive (e.g. blood pressure measurement; blood sampling) and after they have been humanely killed (e.g. taking hearts and blood vessels to examine by microscopy).

Specifically:

Animal Ageing - Animals may be aged up to 2 years for normal ageing, or up to 6 months for prematurely ageing genetically altered lines.

Bone marrow transplant (BMT) - Mice will be irradiated followed by intravenous (into veins) injection of bone marrow from normal or genetically altered mice. This enables mice to have a different mutation in BM-derived immune cells vs non-BM cells.

Mini Pumps/pellets - Implantation under the skin (back) of a miniature dosing pump or pellet, which reduces the need for repeated injections.

Gene activation - Administration of drugs that cause a gene to be switched on or off. This enables analysis of the functions of a gene.

Cell ablation - The depletion/removal of specific cell types by giving drugs that induce cell death. This enables analysis of the functions of a cell type.

Hydrodynamic tail vein injection (HDTV) - A rapid high volume (~3ml) intravenous injection (into veins) that causes DNA to be internalised by cells in tissues, leading to production of proteins.

Substance administration - Administration of drugs that change vascular disease, via diet (food), drinking water, intravenous (into veins), oral gavage (into stomach), nasal (up the nose), subcutaneous (under the skin), intramuscular (into the muscle), intraperitoneal (into the cavity around your stomach/intestines, liver, kidney), pellet, minipump or topically (on the skin).

Peritonitis - Administration of drugs that induce inflammation of the cavity surrounding the intestines/stomach/liver/kidneys/etc. This is a simple way of activating an innate immune response.

Immunisation - Injection of an animal with a protein to induce an immune response with production of antibodies that specifically recognise and react against the protein originally injected. This is a simple way of activating an adaptive immune response.

Diet manipulation - Feeding mice a defined diet such as normal rodent food, or high fat/salt food. This is a simple way of inducing disease

Surgical procedures - Surgery performed on mice to cause a change to normal bodily function or to induce a disease. These include artery injury, artery blocking, or heart injury.

Fasting - Removal of food, but not water, for 6-16 hours. This is needed to enable accurate measurement of blood lipids.

Blood sampling - Removal of blood from a vein, allow subsequent analysis to determine if a disease state is achieved or a drug treatment is working.

Blood pressure - Blood pressure measurement using a tail-cuff (akin to the arm-cuff used to measure BP in humans). This enables disease and/or drug response monitoring.

Measurement of body temperature - Measurement of internal body temperature by putting a thermometer up the mouse's bum. This enables disease and/or drug response monitoring.

Electrocardiogram (ECG) - measurement of the electrical signals that cause the heart to beat via electrodes placed on the skin. This enables disease and/or drug response monitoring.

Metabolic testing - Testing how the body deals with a large amount of sugar or fat, typically after injection of sugar/fat into the stomach, followed by blood sampling. This enables disease and/or drug response monitoring.

Imaging - Imaging the inside of animals without having to put anything inside them (i.e. non-invasive, no surgery) using ultrasound, magnetic resonance imaging (MRI), positron emission tomography (PET), computerised tomography (CT) or fluorescence (glow-in-the-dark). This enables disease and/or drug response monitoring.

Anaesthesia - giving drugs to make the animal sleepy and not feel pain or move during, e.g., surgery. Animals could be anaesthetised for surgery, allowed to recover, and then anaesthetised, say, a week later to perform imaging.

Dosing/Sampling routes:

We will use standard dosing volumes, frequencies and routes, as guided by NC3Rs:

Route	Daily vol. ml/kg	Max. No./day	Max. No. of doses.
oral gavage	20 ml/kg	2	30
intraperitoneal	20 ml/kg	1	30
intravenous	10 ml/kg	1	14
intramuscular	10–50 µl	1	6
subcutaneous	20ml/kg	2	24

Exceptions to this would be thioglycollate IP, which needs to be given in 1ml (standard practice), and the HDTV injection.

Number of steps animals undergo/Cumulative harm:

A typical protocol (~90% of mice) would be one intervention, induction of disease, and 1-2 forms of disease monitoring - e.g. irradiation and bone marrow transplant (BMT), with recovery for ~4 w;

surgery to injure an artery; blood sampling and/or imaging of blood vessels; terminal (euthanasia) procedures to collect tissue and samples.

A worst case scenario (10%) would involve more steps - e.g. BMT, with recovery; Drug injection to switch on genes; repeated injection of another drug; surgery to injure arteries; blood sampling; blood pressure measurement; imaging of blood vessel walls; terminal procedures to collect tissue and samples.

Importantly, the combination of steps under this protocol will always be kept to the minimum possible to directly answer the scientific question (i.e. never a 'shotgun' approach) and will not exceed the severity level.

In particular, an animal will not undergo more than 3 optional steps that may cause more than transient harm or distress. In addition, an animal will never undergo a step that may cause more than transient harm or distress, if it had not already recovered from a previous step.

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

The majority of procedures conducted will only cause transient harm (<5mins; e.g. an injection) with most work mild/subthreshold (e.g. high fat feeding for atherosclerosis studies).

However, to study heart attacks mice have blood vessels to the heart blocked surgically to induce a heart attack. This will cause pain and distress to the animal, analogous to what humans experience during a heart attack. However, the duration of this is less than a few hours and with extensive monitoring to ensure pain and distress subsides and does not exceed severity. Unfortunately, these clinical signs are part of the disease being studied, and it is not possible to study heart attacks without the mouse undergoing a heart attack. Some mice (10-15%) may die of the heart attack, and this death is sudden (as occurs in humans having a heart attack) and very difficult to anticipate.

Similarly, to study aneurysm formation (the bulging of weakened arteries) and rupture (the bursting of bulging arteries) mice need to have aneurysms form and rupture. Mice tend to be unaffected by having an aneurysm in a blood vessel, but rupture leads to sudden blood

loss and instantaneous death. As rupture is the key event that kills humans, it is important to study why some aneurysm don't rupture, whilst others do.

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 = 76%
Moderate = 21%
Severe = 3%

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 09 November 2028

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?

Cardiovascular disease (CVD) is caused by multiple factors, with multiple cell types, tissues and bodily processes playing key roles in its development - and often with considerable interplay between these elements.

Hence, although individual elements of CVD can be modelled in a 'test tube' (e.g. recruitment of immune cells; response to lipid loading), the only way to study the importance of 'test tube' findings on CVD is by disease modelling in animals, with all the multiple cell types, interactions and biological processes occurring in concert.

For example, we can develop a drug that completely blocks a disease-causing function of an immune cell in a 'test tube', but using this drug in an animal may inadvertently block a vital function of the immune cell that is also needed to stop you getting an infection and dying.

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

We consider the use of animal experimentation as the final step in the research programme. Thus, when we use animal models this will be because we typically have

several years worth of 'test tube' experiments that support the use of animal experiments for disease modelling.

If there were non-animal alternatives we would of course use these first, but would again come back to proper disease modelling as the final step.

Why were they not suitable?

There are currently no non-animal alternatives to model the multifactorial elements of CVD.

A retrospective assessment of replacement will be due by 09 November 2028

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 using mice to study cardiovascular disease for >20 years and thus have a great deal of experience and previous data on the model systems we use.

With this experience and previous data we can use statistics to formally test that we are using the correct number of mice to ensure that experiments are undertaken as robustly as possible.

The majority of animals predicted to be used in this project (12,000) are on protocols that will be of a mild or subthreshold severity.

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

We follow the PREPARE guidelines and the CAMARADES/NC3Rs systematic review facility recommendations for the design and analysis of our experiments. We also use the CAMP (conditional allele mouse planner) software to determine the most efficient breeding strategy with complex mouse crosses.

As above, we always utilise statistics before an experiment to ensure the correct number of mice are used to generate meaningful data. We also 'blind' experiments so that the researcher performing or analysing an experiment does not know the mouse was given, and randomly asign mice to a given experimental group. This prevents any unintended bias of a researcher 'wanting' to get a certain result.

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 CAMP (conditional allele mouse planner) software to determine the most efficient breeding strategy with complex mouse crosses. In new systems pilot studies are utilised to ensure the system works as intended with a small group (e.g. 3 control, 3 treated mice) before a full, long-term study (e.g. 15 control, 15 treated mice for a 12 week study).

We also 'blind' experiments so that the researcher performing or analysing an experiment does not know the mouse was given, and randomly asign mice to a given experimental group. This prevents any unintended bias of a researcher 'wanting' to get a certain result.

Spare mice that are not needed (but have not previously been used) are listed on the Establishment's Distribution List, for others with authority to make use of.

A retrospective assessment of reduction will be due by 09 November 2028

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 study various aspects of cardiovascular disease, in particular atherosclerosis (the fatty build up in arteries), heart attacks and aneurysm (the bulging and fatal rupture of blood vessels). We use the current gold-standard models that best represent human disease with the least suffering and distress. We are always actively searching for newer, better models that may come available (e.g. publications, conferences)

Mice are given pain relief before and after surgical procedures, and are monitored frequently to ensure pain and distress never pass a pre-determined threshold at which it would be more humane to euthanise the animal.

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

We only use mice, which represent the 'lowest' model animal that can be genetically manipulated, for which reagents are available to analyse outputs, and which generates human-like cardiovascular disease in a suitable time frame.
For example, to generate experimental atherosclerosis, mice are fed a high fat diet for ~12w. Thus, we cannot use immature 'pups'.

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

The number of steps in a given procedure is minimised to provide the answer to the question being asked with the minimum distress and suffering - i.e. we never use a 'shot-gun' approach of measuring everything 'just in case' something interesting happens. However, for terminal (euthanasia) processes we would recover as many relevant tissues and organs as possible to maximise information output.

Mice are monitored at a frequency in line with the potential for pain and distress caused by the procedure undertaken - i.e. more frequently after surgery - and are never allowed to pass a pre- determined threshold at which it would be more humane to euthanise the animal.

Where appropriate mice are habitualised and/or acclimatised to a new process and/or environment before a procedure is undertaken. For example, all mice undergo a full 7 day acclimatisation period on arrival in the facility. Mice would also be habitualised to a new restraint before, e.g., BP measurement.

For feeding of a tamoxifen diet, mice initially dislike the taste and lose weight, but have no other clinical signs. Thus, we add strawberry nesquick to improve taste, use a body condition scoring sheet to determine that weight loss is indeed caused by food aversion and not something else more serious, and provide mashed food if quick weight gain is needed.

Post-surgery refinements include lowering drinking spouts, providing mashed diet and extra bedding - in addition to general constraints. Specifically, mice will be given appropriate analgesics after surgery to alleviate pain, but the agent chosen will need to not interfere with the parameters being studied -
e.g. an anti-inflammatory analgesic would interfere with immune cell function. However, where possible an alternative class of analgesic would be given (e.g. an opioid).

Where male mice have been singly housed and need to be re-grouped (which can cause fighting), pre- exposure to soiled bedding for several days prior to regrouping can reduce aggression, along with additional monitoring of cages for fighting.

Finally, use of 'mini pumps' for drug administration reduces the amount of handling and stops the need for repeated injections.

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

As above, we follow published best practise guidelines, such as ARRIVE, PREPARE, LASA Aseptic surgery guidance and NC3Rs recommendations, to ensure optimal planning, reporting and refinement of animal experiments.

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

As above, we are always actively searching for newer, better models that may come available (e.g. publications, conferences). More formally we follow the ARRIVE guidelines and keep up to date with other developments and advances in resources such as the NC3Rs (https://nc3rs.org.uk/our- portfolio),the North American 3Rs collaborative

(https://www.na3rsc.org/the-3rs/), the Norecopa database (https://norecopa.no/), and the Danish 3R-Centre (https://en.3rcenter.dk/), with the aim to implement important developments and alternatives swiftly.

A retrospective assessment of refinement will be due by 09 November 2028

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?

8. Understanding the Role of Inflammation in Stroke Development

Project duration

3 years 0 months

Project purpose

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

Key words

Stroke, Immune cells, Platelets, Inflammation, Blood cells

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

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 aim of this project is to explore the role of inflammation in the development of stroke.

A retrospective assessment of these aims will be due by 26 October 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?

Stroke is the second largest cause of mortality worldwide. In addition, stroke causes severe disability in those patients who survive, making them sometimes unable to normally move, eat and talk. Stroke develops when a blood clot suddenly occludes a vessel in the brain and limits the delivery of blood to the tissues (this process is called "ischemia"). There is only one specific treatment of stroke, it is called tissue plasminogen activator (tPA). This drug dissolves the clot in brain vessels restoring blood circulation and oxygen supply to neurons. However, tPA can be administered only within a very limited time frame (about 3 h) after onset of symptoms, which is impossible in most of the cases. Also, tPA has multiple deleterious side effects, for example, it can cause blood brain barrier disruption and lead to brain hemorrhage. Moreover, another stroke can follow the first event if platelets and other cells remain abnormally active.

Inflammation developing as a result of tissue death following stroke is another strong complication of stroke. Inflammation is recruitment of immune cells, such as leukocytes, in the blood vessels, migration of the cells to the brain tissue where they accumulate and inflict further damage to neurons, key cells responsible for our ability to undertake functions necessary in life. This does not happen under normal circumstances and inflammation commences only once areas in the brain become re-perfused with blood as circulation is restored following the stroke. Inflammation expands the dead tissue area leading to further deterioration of patient's condition even when the clot is already removed (so called, reperfusion injury). Treatment abilities of cerebral inflammation induced by stroke are limited. Thus, it is important to urgently find new approaches to suppress inflammation i.e., recruitment of leukocytes, platelets and mast cells and limitation of their destructive effects. This will allow to save lives of stroke patients, improve their quality of life and decrease heavy burden on health systems around the world. Overall, the central goal of this study is identification of new inflammation-related mechanisms of stroke that can be targeted to minimize tissue injury and neurological deficit.

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

This work will generate **new fundamental knowledge** on the role of inflammation-related cells and platelets, individually or in combination, in ischemic stroke. This will increase our understanding how this debilitating and life-threatening diseases develops and provide new insights into basic mechanisms of post-stroke inflammation in the brain.

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

The beneficiaries of the project will be:

1. The immediate short term beneficiaries of this project are colleagues and collaborators working in this field of research. Fundamental data obtained in these studies will enrich our state of knowledge in the field and trigger and fuel further research in this direction; this is an immediate benefit that will become clear already during the course of project realization/PPL;

The following beneficiaries will likely benefit from the results of this project at the timescale beyond the lifetime of this PPL:

2. Medium-term beneficiaries:

Scientific community worldwide, dealing with stroke research. This will become possible as a result of publication of research papers and presentation of the results at scientific meetings and conferences.

2. Long-term beneficiaries:

Clinical practice: New fundamental pathways regulating post-stroke inflammation will be identified, which will pave the way to target them therapeutically in a more flexible and personalized fashion.

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

The results will be published in professional/scientific journals and shared with public mass media as much as possible. The data will be presented in both national and international scientific conferences. I will collaborate with world leading experts in the stroke field to ensure highest quality of the work and presentation of these results in these establishments and around the world. Importantly, I will make a special effort to publish even negative data, which is very important as it saves resources and animal lives that would otherwise have been wasted in an effort to perform the same experiments.

Species and numbers of animals expected to be used

- Mice: 2100

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 have been chosen because: 1) the anatomy of cerebral vasculature keeps developing throughout earlier developmental stages (e.g., embryo or neonates) with the majority of its anatomical entities (e.g., circle of Willis with connections to key vessels including middle cerebral artery) obtaining its final shape in adult animals; 2) stroke predominantly affects adult people and therefore the use of adult mice will better recapitulate vulnerable age in humans.

There is no one animal model that recapitulates all aspects of the human condition. We aimed to choose a model, which is least traumatic to the animal and generates most reproducible results. In addition to these considerations, the model that we have chosen is particularly suitable for studying post-stroke inflammation-related alterations of the brain due to the following reasons: 1) vast majority of the existing preclinical data on mechanisms of stroke has been generated on mice and therefore the use of the same species is necessary to, firstly, appropriately plan experiments basing on existing information, and secondly, to be able to correctly incorporate obtained results into current state of knowledge on the topic; 2) mice the least sentient species that display suitably human-like anatomy of brain vessels; 3) mechanisms of stroke explored in mouse models are largely applicable to the human disease, which makes the work clinically relevant; 4) techniques of modeling stroke in mice are very well-developed and therefore number of required animal as well individual animal suffering can be minimized; 5) mice are the only

species for which there is a wide range of genetically altered strains already available, which makes mice an ideal model to study roles of separate molecules (e.g., receptors, signaling molecules etc.) in stroke.

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

Stroke will be induced under anaesthetic by introducing a filament into an artery in the brain. This causes local loss of blood flow (ischemia), causing a stroke, the degree of which is dependent upon the length of time that the filament is in position. The filament will then be removed, and blood flow returns to the area (reperfusion). Before or during ischemia or reperfusion period, mice may be administered different drugs or chemicals affecting the immune/inflammatory component of the stroke pathogenesis, or cells participating in the inflammatory response to explore their role in post-stroke recovery. Throughout the experiment, neurological score may be evaluated. This means several simple tests checking, for example, general mouse behavior in the cage or how quickly the mouse can get rid of a sticky tape on its paw etc. This is necessary to evaluate the degree of tissue damage, and whether the administration of drugs/compounds/cells has improved brain function. Mice will be closely monitored and in case of any unexpected events, the animal will be humanely killed. The mice will receive pain relief to deal with surgical-associated pain. The number of animals used in experiments will be limited to minimal possible based on a specific statistical procedure called "power calculation".

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

The main adverse effect is abnormal "circling" of the mouse when the animal, instead of walking straight, tends to make circles in the direction opposite to affected hemisphere (i.e., if the stroke is induced in the right hemisphere, the mouse will tend to turn to the left). This recapitulates abnormalities, which human patients experience following stroke when extremities on one part of the body function worse than on the other. The neurological deficit will remain within short-term experiment (24 h reperfusion) and may improve in longer experiments. Animals can also lose about 10-15% of their weight within first 24 h after the surgery, which again reflects similar changes seen in humans with the disease. There is a tendency for the animals to regain the weight after around a week. Most of the experiments will be short-term (up to 24 hours). Mice will be provided with pain relief when 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)?

50% mild

0% moderate

50% 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 October 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?

Brain is a very complicated organ with multiple regulatory contours of its blood supply and variable susceptibility of different brain cells (e.g., neurons, neuroglia etc.) to low oxygen levels, which underlie stroke. In addition to local environment in the brain itself, multiple systemic factors, such as blood pressure, vessel tone, hormones, and other biological regulators, take part in maintaining the healthy state of brain tissue and counteracting pathological stimuli. The entire complexity of conditions in the whole organism, which constitute both causes and consequences of stroke, cannot be recapitulated in vitro (i.e., in a test-tube). This complexity includes hyperactivity of blood platelets, inflammation caused by other blood cells, interaction of blood cells between each other and the vessel wall, shear conditions created by the flowing blood, age, accompanying diseases, initial and late responses of the vessel wall in the brain, integrity of the blood-brain barrier, individual features changing from person to person and many other factors that, in combination, cause the disease and define its further course. Only taking all these factors as a whole can allow to create conditions similar to those of stroke in humans. Moreover, in addition to delineating mechanisms of the disease, identification of new targets to prevent and/or treat it requires the use of a model that would recapitulate real conditions and circumstances as close to actual situation as possible. This is why the use of animals in such a project is necessary to successfully develop new strategies to prevent and treat stroke.

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

Several non-animal-based approaches are not only considered but will be actually used to replace animals or at least minimize their number. These approaches allow to investigate separate features of brain cells under the influence of isolated factors typical for stroke. For example, the integrity of blood brain barrier can be tested using cultured brain vascular cells that can be commercially purchased. In this method, cells are grown on a special surface called the Transwell system, then a tested drug is added to the cells and then their permeability is determined. Another alternative method is checking of susceptibility of neurons (brain nerve cells) to hypoxia - lack of oxygen resulting from insufficient blood supply in stroke. This can be addressed by placing cultured cells into a hypoxic incubator in the presence or absence of tested drugs, and checking their properties afterwards. These and other in vitro (in a test-tube) approaches will be used for two purposes: 1) high-throughput screening of drugs affecting blood cells or blood cells themselves on neurons, when multiple experimental conditions should be checked, which excludes the use of animals at the stage of choice of single drug, drugs, manipulations, factors or conditions that will be verified already in mice 2) exploration of fine mechanisms of the influence of blood cells, hypoxia or other factors on brain cells 3) we will attempt to build a

computational model of blood cell egress and accumulation in the infarcted brain and predict the role of particular mechanisms/receptors/molecules in this process, and 4) we will analyze human stroke thrombi for bone marrow-derived cells and their components.

These alternative approaches will allow to minimize the number of experimental animals that will used only and exclusively when in vitro methods cannot provide the required information.

Why were they not suitable?

As mentioned above, these alternative methods do exist, have been considered and will be utilized to obtain any piece of information that can be achieved without use of animals. Although in vitro methods cannot completely replace the use of animals due to considerations stated above, they will be first choice whenever value and reliability of results obtained in them is equal (let alone when exceeds) to those expected to be obtained using mice.

A retrospective assessment of replacement will be due by 26 October 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 used per experiment has been evaluated based upon: 1) literature data on the topic; 2) in-house data, 3) personal communications with several labs conducting stroke research around the world, and 4) Experimental Design Assistant (EDA), a free software developed by the NC3R.

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

Wherever possible, experiments will be performed blindly, which means that the person performing the experiment will not be aware of the administered treatment or mice genetic background. Also, where possible, proper randomization of animals in experimental groups will be implemented. This approach allows to obtain more objective results and therefore reduce the number of required animals. Efforts will be made to use the same animal to answer several scientific questions simultaneously, for example, tissues obtained from the same mouse will be used to measure different biological parameters thus limiting the necessity to perform additional experiments and use more animals.

When possible, animals will be used as their own control. For example, if changes in some parameters of blood or blood cells resulting from stroke are to be investigated, where there

is not an undue impact on the welfare of the individual animal, a blood sample will be taken from the same mouse prior to stroke induction and after that. This approach will not only allow to exclude the use of a special control group of mice (which is a substantial reduction by itself), but also decrease numbers of mice in the experimental group because statistics requires much lower numbers for comparison of data obtained from the same animal (i.e., with minimal or even zero variation). In longer experiments (> 24 h), neurological status will be evaluated in the same mouse throughout the experiment. Thus, all the data will be obtained in the same experimental group without need to use separate groups for different time points.

Where possible, a statistical method called "Resource Equation" will be utilized to plan the experiment. This method allows to diminish the number of required animals to minimal.

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

All experiments will be well planned ensuring that efficient breeding strategies are used to minimize wastage. All mice will receive identical post-operative treatment to minimize effects of environmental

factors and thus improve reproducibility of the results. Mice in experimental groups will be comparable in age and weight to minimize inter-individual differences (noise). For the same purpose, inbred (i.e., genetically identical) mice will be employed. Where possible, cell culture of neurons or glia (auxiliary brain cells) cells or their co-culture will be utilized to explore effects of stroke conditions such as hypoxia (oxygen deprivation) and to test the impact of different treatments to brain cells (e.g., blood brain barrier integrity). In these cases, animals will be either completely replaced by an in vitro method, or the number of required animals will be greatly reduced and limited to just verification of selected results of in vitro screening. All the data will be constantly monitoring for any mistakes and appropriate statistical methods will be used for analysis.

A retrospective assessment of reduction will be due by 26 October 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 plan to induce stroke in mice by inserting a filament filament into an artery in the neck, then progressing it forward so as to occlude an artery in the brain. There are other

published methods available that we have declined to use currently as they do not provide any additional scientific benefit and represent less refined approaches:

- Electrocoagulation (coagulating the blood and destroying the structure of the artery), and chemical or photochemical methods (inducing a thrombus in the common carotid artery and dislocating it into the middle cerebral artery) all carry a high likelihood that the clot will move to an undesirable position, or multiple clots will form, both of which are hard to control. The adverse effects upon the animal are therefore hard to predict, and the results may not be useful scientifically.

- Pharmacological methods (e.g. applying vasoconstrictor substances directly onto the artery or injecting it into neighbouring tissue to induce prolonged, local ischaemia) are unlikely to result in real stroke, rather prolonged ischemia of brain tissues, which is less clinically relevant and the results are highly variable due to individual differences between animal reactions. The low clinical significance of the model is due to inability to control the duration of ischemia and its transformation to reperfusion.

- Production of thrombus directly on the MCA through a hole in the skull requires drilling of the skull, which is a more invasive route than introduction of a filament via an artery in the neck as per the model of choice in this PPL.

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

Mice, are widely used in pre-clinical/translational stroke research worldwide because they have brain anatomy similar to that in humans. The structure of brain vessels (common, internal and external carotid artery, MCA, architecture of the Circle of Willis) is the same as in the human brain. Moreover, neurological response to stroke recapitulates well symptoms observed in patients. Other species used in biological research either have absolutely different anatomy very far from human brain (for example, flies or Zebra fish), or are higher species than mice (for example, rats, guinea pigs, rabbits, dogs etc.). The same relates to more immature life stages because embryos and very young mice (e.g., 1 week old) have only developing brain vascular system and cannot therefore be used in mimicking stroke.

The reperfusion phase of minimum 24 h is necessary for the stroke to develop. This makes use of terminal anesthesia impossible because the animal would not endure such a long time of anesthesia.

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

Mice will be handled using refined handling techniques, and bespoke welfare score sheets will be used to monitor animals for emerging adverse effects. Animals will be monitored closely, particularly immediately post-surgery to ensure animals are not suffering unexpected adverse effects based upon information from the NC3Rs documentation. During and after surgery, animals will be kept warm, which has been shown to help animals used for this particular model to recover better.

Prior to surgery, animals will be acclimatised to water-containing gels and food on the cage base, as both will be provided to help support the animal post-stroke.

The animal may also receive a subcutaneous injection of saline as this has been found to aid recovery post-stroke. Analgesia, soft bedding, group housing, increased enrichment may also be implemented.

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

Maximum frequency of blood withdrawal and maximal volume of blood collection will not exceed LASA guidelines. To ensure appropriate formulation and planning of the study, choice of appropriate experimental design and statistical methods, and to reduce experimental bias, Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines will be employed. The results obtained within the framework of this project will be published in high impact scientific journals conforming with the ARRIVE guidelines provided by NC3Rs (https://www.nc3rs.org.uk/sites/default/files/2022-01/The%20IMPROVE%20Guidelines%20%28poster%29.pdf).

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

I will use the NC3R recommendations specifically for the stroke model (https://www.nc3rs.org.uk/sites/default/files/2022-01/The%20IMPROVE%20Guidelines%20%28poster%29.pdf) to inform my study design and ensure animals are provided with appropriate and effective support measures, regularly and thoroughly review the NC3R web site https://www.nc3rs.org.uk/, and implement any new requirements or recommendations that would appear during this project. Moreover, I will make sure that my research team is well aware of the news in 3Rs, which will be discussed in our lab meetings at least once a month. I will work in tight connection with our animal facilities to exchange any new information and implement new approaches to refinement. I will also monitor professional literature in the field to learn potential new statistical strategies to reduce the number of mice required for every experiment.

A retrospective assessment of refinement will be due by 26 October 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?

9. Phenotyping Malaria Infection in Different Organs

Project duration

5 years 0 months

Project purpose

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

Key words

Malaria, Plasmodium, Transmission, Imaging, Sequestration

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.

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?

To improve our understanding the transmission biology of Plasmodium parasites which cause malaria. Transmission biology involves the development of sexual stages of the parasites (gametocytes) from asexual forms of the parasite within a mammalian host, and their uptake by biting mosquitoes.

A retrospective assessment of these aims will be due by 05 December 2028

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?

Malaria kills 600,000 people a year, infects around 250 million people annually and is one of the deadliest diseases in the world. Interrupting transmission between people and preventing further cases is thus of great interest.

Gametocytes are the only stage of the parasite life cycle that can develop in the mosquito and lead to the generation of new parasites. These new parasites can be transmitted to a new host if an infected mosquito bites another person.

Asexual and sexual stages of the parasites reside in the blood of infected people, but also in deep tissues such as the bone marrow and spleen. The gametocytes must also be present in the blood vessels of the skin, in order for a biting mosquito to ingest them. It is important to research how, why, and where the parasite interacts with the host in these different organs. A greater understanding of how gametocytes are generated and transmitted to mosquitoes is key to helping preventing transmission of the parasites.

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

This study will provide vital new information and evidence in an area of malaria research that is under- researched. Our data will be published in scientific journals and pre-print repositories in the form of publications and as well as being presented at conferences. In addition to generating new knowledge, we also aim to develop new technologies for imaging parasites in different organs of mice, which we hope will aid in the development of anti-malaria therapeutics.

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

The development of new imaging methods will benefit us by allowing us to view the parasite in organs that has either never been attempted before or where there is little data at present.

We will spread our new acquired knowledge and scientific advances to the rest of the malaria and wider scientific community.

Advances in our study will hopefully help in the development of therapeutics or methods to reduce the number of cases and deaths of malaria in the world.

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

This study will seek to increase collaboration by working with groups here at the establishment as well as with groups in institutes in the UK and abroad. It is important that we publish evidence as soon as we can, from both successful experiments and unsuccessful experiments to inform the scientific community. This will be done by posting on pre-print servers which are widely viewed now as a great tool for producing transparent science. By posting on these servers, prior to peer-reviewed journal articles, a greater proportion of people can view our work, which may generate new collaborations and further improve the quality of our research.

Species and numbers of animals expected to be used

- Mice: 3,000 mice

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 (from 6 weeks of age) as they are a great model for studying malaria, with many of the same processes that occur during human disease also occurring in malaria mouse models. We intend to use several different species of rodent-infective Plasmodium parasites, with a number of different strains of mice. Using different combinations of Plasmodium species and mouse strains allow different aspects of malarial disease to be explored, with the most suitable models reflecting best what happens in humans. We are primarily interested in the transmission of parasites between mice and mosquitoes. Successful transmission of parasites from infected mice to mosquitoes has previously been demonstrated and therefore we suggest that the biological processes in infected humans and mice, prior to transmission, are the same or very similar. Malaria transmission can occur in humans with no symptoms of the disease and thus we aim to model this, with limited interest in severe disease and associated pathology. As such, we will be using combinations that do not lead to Experimental Cerebral Malaria, a symptom of severe malaria which can lead to death in humans and experimental mice.

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

Most of the mice will be injected with malaria parasites, either through a vein in the tail or an injection in the abdomen.

We follow the infection of the mice by taking tail pricks. Tail pricks involve puncturing the tip of the tail to acquire a small amount of blood which we use to make a smear on a microscope slide, before staining in the laboratory to count the number of parasites in their blood.

Later, usually within a week, the mice will be anaesthetised and undergo surgery to expose their organs e.g. skin, bone marrow or spleen. Whilst anaesthetised, these organs will be imaged using a microscope to observe the parasites that reside there. After imaging is completed, mice will be humanely culled.

Other mice will be infected with parasites and after a few days, be killed and have their organs harvested for analysis without undergoing imaging under anaesthesia. Where we want to acquire blood, infected mice will be anaesthetised and then blood removed from the animal, before completing the humane culling by confirmation of death by breaking the neck.

At other times we will administer vaccines, substances that modulate the immune response (immunomodulatory substances), agents that allow for the expression of specific genes (gene induction) or drugs to manipulate the host or parasite. These manipulations will allow us to see if certain host/ parasite processes play a role in the location and behaviour of the parasite or if they affect parasite transmission to mosquitoes. Any novel

substances will be tested in advance in small-scale studies on a few mice to check that there is not any issues with toxicity.

As such at times, Plasmodium-infected mice will be used to infect mosquitoes and continue the parasite life cycle, by allowing mosquitoes to feed on them. This is a critical part of our study into transmission of the parasites. During these procedures, the mice will be anaesthetised.

Other mice will be used to blood feed mosquitoes (on a weekly basis), which is an important requirement for keeping a healthy mosquito colony. Female mosquitoes require the proteins within mouse blood to be able to make their own eggs and mosquito offspring. These mice will be anaesthetised and placed on top of a cage of mosquitoes. After the mosquitoes have fed the mice will humanely culled.

Single housing of animals will be avoided where possible.

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

In most of the procedures the mice will suffer mild, temporary discomfort following injections or tail pricks for blood sampling. The more painful procedures, such as surgery and mosquito feeds, are performed under anaesthesia, to ensure the mouse only experiences minor discomfort and no pain. After the imaging, mice will be humanely culled rather than allowed to recover and returned to the housing facility. Only in two situations: when the ears have been imaged (which doesn't require any surgery) or where whole body imaging is performed are subsequent imaging sessions allowed on the same mouse. Here mice will be allowed to recover and returned to the animal facility. Repeated imaging of the ear pinna is capped at four sessions, with at least a 24-hour gap between sessions to allow recovery and prevent weight loss which is associated with receiving repeated rounds of general anaesthetic. Whole body imaging is capped at 20 whole-body imaging sessions (twice daily maximum, and a maximum of five sessions over a seven-day period).

We will predominantly use the parasite Plasmodium berghei for infections, but we will use mouse strains that are not susceptible, and do not develop Experimental Cerebral Malaria (ECM), a condition that results in convulsions, coma, and death. Nonetheless, no strain of mouse can control Plasmodium berghei infections and, if left untreated, mice would eventually die from a high parasite burden in the blood. However, we will be able to perform our studies before this occurs, successfully acquiring data before the mice suffer any severe ill-health from the infection. If ill-health does occur, the mice would be humanely culled immediately to prevent further suffering.

We also intend to use Plasmodium chabaudi infection infrequently, which can lead to waves of high parasite burden in the blood and the onset of anaemia which can make the mice sick and lose weight. However, the mice are able to control the infection within 72 hours of the height of infection, and then do not go onto suffer further ill health.

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)?
95% mice infected with Plasmodium berghei should only experience mild, transient distress from injections. However, there is the rare occasion when a mouse will become

poorly for reasons unknown, or potentially as a side effect of procedures undertaken for imaging purposes or infection by mosquitoes, and so 5% of mice infected with Plasmodium berghei may experience moderate harm including signs of lassitude (reluctance to move, isolation and failure to groom).

Those mice infected with Plasmodium chabaudi or Plasmodium yoelii will for the most part experience only mild distress (75%) from injections. 20% may experience moderate harm as a result of high parasitaemia (<10% weight loss, anaemia and some lassitude (reluctance to move, isolation and failure to groom)). But some mice may experience severe adverse effects (5%) as a result of high parasitaemia (weight loss of 10-15%, persisting isolation, and persisting reluctance to move or groom). These effects of P. chabaudi infection are transient however, and they should recover from any ill- health.

Aside from the effects of Plasmodium infection, those animals undergoing surgery are not expected to experience distress, as the procedures will be administered under terminal anaesthesia.

Those mice undergoing mosquito feeds to maintain a mosquito colony will not be used for other experiments before or after feeding and thus will be placed under a non-recovery limit.

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 December 2028

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?

Studying rodent infective species of Plasmodium is beneficial as it gives us access to all the life stages of the parasite (including liver-stages and blood stages in the mouse) as well as those in the mosquito midgut and salivary glands. Having access to all these life stages is not possible with Plasmodium species that infect humans, especially those life stages that infect deep tissues, which are inaccessible by non-invasive methods. Thus, as we are most interested in parasite residence, development and transmission in a number of deep organs, namely those of the haematopoietic niches e.g., spleen and bone marrow, as well as the skin, it is necessary to use mice and mosquitoes in this project to visualise what is happening at an organ level.

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

The use of membrane feeders has been used to investigate the uptake of gametocytes by mosquitoes. The feeders contain human red blood cells infected with Plasmodium, which were generated by laboratory culture. The blood is then fed to mosquitoes.

There has been some development of culturing systems that mimic organ environments, which could be used to investigate interactions between the parasite and cells of different organs.

Why were they not suitable?

Membrane feeders do not help answer questions about where the parasites are in the skin. There are differences between the feeding of mosquitoes on membrane feeders compared to feeding on the skin of infected individuals, with mosquitoes usually becoming more infected when fed on skin (doi:10.1371/journal.pone.0042821; doi:10.1172/JCI98012). This could be for a variety of reasons. To understand what really occurs during skin feeding, which is how malaria transmission occurs in nature, a mouse model would be best to help us to research this.

To date, the development of organ culture systems is not sufficiently advanced to allow us to realistically achieve our goals. However, we have plans to develop culture systems for eventual use in trial experiments before we confirm the results in mice, thus reducing the number of mice needed.

A retrospective assessment of replacement will be due by 05 December 2028

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?

Experiments were planned very carefully to estimate and minimise the number of mice that we would need to use. Statistical analysis, performed in advance, allowed us to determine the minimal mice that can be used to achieve conclusive answers for our individual experiments.

The estimation includes mice used in pilot studies, the main experiments we would perform to acquire meaningful data, and mice needed to maintain a mosquito colony.

We will use approximately 500 mice a year for imaging and maintaining parasite stocks and about 100 mice a year to maintain the mosquito colony.

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

We used NC3R's Experimental Design Assistant to determine the minimum number of animals required to give meaningful and solid statistical results, with guidance from a biostatistician on how best to design the experiments.

The core use of mice in this project will be for imaging. As we can acquire many images and data from one mouse, then less mice are needed to acquire sufficient amounts of data.

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, organs will be harvested from mice after they have been imaged, so that their tissues can be used to optimise or perform other experiments or share organs with others to minimise the number of mice required. Pilot studies, particularly imaging, will be performed first on culled mice then on individual live mice to optimise procedures before designing a large experiment of 4 or more mice per condition. We plan to develop a cell culture system which mimics the blood system in different organs and when developed, we plan to use this first in trial experiments before using mice.

We do not intend to routinely use genetically modified mouse strains, however if required, we will seek existing colonies for use rather than generating our colonies to reduce the number of mice being bred.

We will use both sexes of mice, to counter any variability between sexes as well showing that our results are generalisable. We do not expect there to be any difference between sexes, this in this way, less animals would be required to make the same statistical judgment, even if a difference is found between sexes.

We will not set up a breeding program, but instead buy in our mice as and when required to reduce the number of surplus animals being bred unnecessarily. We will collaborate with other groups to use any mice that they do not require to prevent unnecessary euthanasia of healthy animals.

A retrospective assessment of reduction will be due by 05 December 2028

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 mouse models that allow access to all stages of the parasite life cycle. Some mouse strains can develop a phenomenon called experimental cerebral malaria (ECM) when infected with the species Plasmodium berghei, leading to convulsions, coma, and death. In this project, we will only use mouse strains that do not develop ECM when we infect with Plasmodium berghei, as the progression of ECM is not of interest in this project.

A small proportion of mice in this project will be infected with Plasmodium chabaudi or Plasmodium yoelii, but we will only use strains of mice that do not succumb to lethal infections. We will also only use strains of parasites that cause transient high levels of parasitaemia, resulting in anaemia and weight loss, but from which the mice recover as they control and clear their infections

The majority of the mouse-parasite combinations we will use will be combinations that are well studied in the community and thus we know a lot about how the parasite behaves in these mice as well as the expected trajectories of illness.

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

The species of Plasmodium we will use only infects rodents and thus a less sentient animal could not be used to research these parasites that model human infection. We cannot continually culture the rodent species Plasmodium berghei, Plasmodium chabaudi or Plasmodium yoelii in the laboratory and thus require living rodents to generate them. However, the proposed surgeries in this project will be performed using terminal anaesthesia, to minimise suffering following recovery from the anaesthesia, and reuse.

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

To prevent suffering from adverse effects due to malaria infection, we will monitor mice daily including the weekends (once parasites first appear in the blood). We will check the parasites in their blood and monitor the mice through several score sheets designed to monitor the outward appearance of the mice. This will include grimace scores, responsiveness, posture, paling of skin (due to anaemia), breathing rate and presence of diarrhoea. Mice that look unwell and do not improve rapidly (over one day) will be humanely culled.

Prior to imaging, any surgery will be performed on a heated mat and the microscope stage will remain at 37C to minimise discomfort while anaesthetised. Anaesthesia will be keenly monitored; if any signs of suffering or complications occur, the mouse will be euthanised immediately. The use of genetically modified parasites which glow allows us to easily distinguish them when imaging. Mice will be humanely culled after all imaging involving surgery and not used in repeat imaging, to prevent any stress from recovery. Repeat imaging will only occur where non-surgical procedures have been employed i.e. to image the ear or where whole-body imaging is performed. These will be limited to four ear imaging sessions (at least 24 hours apart), or 20 whole-body imaging sessions (twice daily maximum, and a maximum of five sessions over a seven-day period).

We will work with the NACWO and NVS teams to ensure that the approaches we are taking to imaging remain the most appropriate and effective and develop and refine new methods as they appear. This will include the development of adaptors to microscope stages to maintain the mouse in an appropriate yet comfortable position.

The team involved in this project will be trained sufficiently to be competent in the procedures they will perform and will be assisted by the skilled animal staff at animal facility.

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

We will continue to follow the latest NC3Rs advice on animal welfare and the PREPARE and ARRIVE guidelines on good practice when publishing our work. This is so that other researchers can accurately replicate and reproduce our results where necessary.

We will also keep abreast of the scientific literature including pre-print servers to stay informed of developments in our field of study and improved animal practice when imaging rodent infective species of Plasmodium. We may also learn of other refinements at scientific meetings and workshops.

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

We receive regular updates from NC3Rs through our establishment and we will ensure we stay informed about the latest advances in the 3Rs through the NC3Rs website and 3Rs symposia, also considering how we can implement 3Rs approaches in the context of our project. We will stay in close contact with our NACWO and NVS teams to ensure that the approaches we are taking to imaging remain the most appropriate and effective and develop and refine our methods continuously.

A retrospective assessment of refinement will be due by 05 December 2028

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?

10. Molecular Mechanisms of Tolerance and Immunosuppression

Project duration

5 years 0 months

Project purpose

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

Key words

Cancer, Immunosuppression, Immunological tolerance, Infection,Inflammation

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 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?

Our research aims to discover new mechanisms underlying immunological tolerance and immunosuppression, and to define mechanisms that have distinct rather than shared roles in these processes.

A retrospective assessment of these aims will be due by 20 October 2028

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 normal function of the immune system is required to fight infections by bacteria and viruses while its disordered function contributes to many disease processes including autoimmunity, allergy, chronic infection and cancer. The immune system is composed of diverse cell types that can either promote or inhibit immune activation. While inhibitory components of the immune system are required to suppress autoimmune and allergic inflammation in a process referred to as immunological tolerance, they can also suppress potentially beneficial responses against infections and cancer, in a process known as immunosuppression. Both are important immunological processes and targets for the development of new drugs.

Newly developed therapies targeting mechanisms of immunosuppression have shown promise in activating immune function in patients with cancer, but since the mechanisms of immunosuppression being targeted also contribute to immunological tolerance, a proportion of individuals treated develop inflammation which causes side effects and limits therapy. Discovery of such immunosuppressive mechanisms which do not contribute to immune regulation will enable more specific therapies to be developed, allowing, for example, disruption of cancer immunosuppression without induction of inflammatory disease in patients.

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

Therapies that work by targeting the immune system have brought about significant health benefits on a global scale. For example, therapies targeting immunosuppressive mechanisms in cancer are presently revolutionising the treatment for patients with metastatic disease.

Our research will extend our fundamental knowledge of how the function of the immune system is controlled not only to prevent otherwise deleterious autoimmune and allergic inflammation but also to limit effective immunity against chronic infections and cancer. The research will also provide a basis for the development of new therapies aimed at controlling immune function in patients with a variety of other disorders in which the immune system plays a critical role in inflammatory diseases and infections.

We will publish our research in peer-reviewed journals and present our findings ahead of publication as oral presentations and posters at national and international conferences.

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

Our research will be of benefit to researchers in related academic fields, such as cancer immunology, inflammatory disease biology and infectious diseases, and to researchers in the field of gene regulation and epigenetics. Aside from its relevance to academic researchers, the work is relevant to researchers aiming to make new therapies for individuals with immune-mediated disorders, cancer and chronic infection, including pre-clinical researchers and the pharmaceutical industry, with whom we have established

collaborations.

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

The outputs of our research are primarily disseminated through research publications and presentations. Data and reagents are also directly shared with academic and industrial sector researchers ahead of publication. We also strive to engage the public with our science. We will seek opportunities to secure intellectual property and commercialise our research to foster UK indutrial and bioscience growth.

The unsuccessful approaches will be submitted and pre-printed on bioRxiv (https://www.biorxiv.org/), an online archive and distribution service, for sharing with global scientists.

Species and numbers of animals expected to be used

- Mice: 53780

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 behaviour of the immune system is poorly modelled *in vitro* (in a test tube, culture dish, or elsewhere outside a living organism), due to the complexity of the cellular interactions taking place *in vivo* (in a living organism). Hence we require an experimental system where homeostasis and disease can be modelled *in vivo*. The immune system in mice is similar enough to the immune system in humans that valuable parallels can be drawn. The availability of different genetically modified mice and reagents that recognise mouse cells means this species can be used more efficiently than any other species to ask questions about the role of particular genes in the immune system.

We usually use adult mice because the immune systems of adult mice are similar to humans and our research focuses on diseases which primarily affect adults. Apart from studying adult mice, we also need to study aged mice to investigate the immunoregulatory mechanisms which control inflammageing, an inflammatory condition developed in age.

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

A majority of mice will be used in the breeding of genetically-modified animals for experiments. A proportion of mice will be killed in a humane way so that organs can be analysed within the laboratory. Some mice will be used in experiments to test how the immune system responds to infections, inflammation and cancer. Typically, animals will experience mild, transient pain and no lasting harm from the administration of substances by injection using standard routes (intravenous, subcutaneous, intraperitoneal).

For example, we will test the immune response of mice bearing genetic alterations in the immune system to tumours by implanting small numbers of tumour cells under the skin of animals and letting them grow. Tumour growth will be monitored regularly by trained staff. Animals will not be allowed to suffer excessively, or beyond well-defined criteria. Animals

likely to develop adverse effects that extend beyond the well-defined limits will be killed using a humane method. After killing animals, tumours and organs, such as spleens, lymph nodes and lungs will be taken and analysed in the laboratory to gain insights into how tumour immune responses are suppressed. In general, these experiments take around three weeks from tumour injection to euthanasia and animals may receive immunomodulatory substances during tumour growth to examine the therapeutic potential of targeting immunoregulatory mechanisms. Similarly, we will test the response of animals with genetic alterations in immunoregulatory mechanisms to acute infection, chronic infection, allergic asthma, and experimental autoimmune encephalomyelitis (EAE) using well-established disease models. The typical duration of such disease models is 2 weeks, 16 weeks, 4 weeks and 4 weeks respectively.

We also need to use models of cancer, and autoimmune and allergic inflammation to test immune function in animals which have received immune cells from another mouse. In such experiments, animals will typically receive an intravenous injection of immune cells and then infection, inflammation or cancer will be induced. Animals will be routinely assessed for signs of illness and weight loss and animals likely to breach the severity limits of the protocol will be euthanized.

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

A majority of animals will be used to breed and expand genetically altered mouse strains essential for the research, and will experience no significant adverse effects. Likely adverse effects from experimental procedures depend on the experimental model being used. For example, likely adverse effects in cancer models include the ulceration of tumours and skin damage over the site of injected tumour cells. These adverse effects are expected to last for 3 weeks, with significant ulceration triggering a decision to euthanize the animal. Animals under infection or inflammation models may lose weight and exhibit reduced activity. These adverse effects are expected to last for 2 weeks until the infection is cleared or the inflammation subsides. Animals induced with colitis are expected to have diarrhoea and blood is occasionally observed in the stool. These adverse effects are expected to last for 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)?

Mice mild 92%
moderate 7%
severe 1%

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

- Killed

A retrospective assessment of these predicted harms will be due by 20 October 2028

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?

Animals are necessary to understand how the immune system works because the various interactions of immune cells with other cells and substances in living animals cannot yet be generated in test tubes. Features such as the distribution of immune organs throughout the body and the ability of immune cells to migrate into almost all tissues of the body make investigations of the immune system in the whole animal context essential. Adaptive immunity (the type of immunity which remembers previous exposures), which is the subject of this research, evolved in vertebrate animals and is not present in less sentient organisms. Among vertebrates, many cellular and molecular features are highly conserved between mice and men. Many useful tools and well-established experimental models for experiments in mice already exist, enabling us to perform research using mice in an efficient manner that minimises the number of animals we need to use. Therefore, the use of mice in this research is necessary.

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

We considered using cells cultured and expanded *in vitro* to test whether certain molecular pathways have an important role in controlling the immune system. These experiments while useful in replacing the need to use animals at the early stage of discovery, are very poor at modelling how the immune system works within the body.

Why were they not suitable?

In vitro experiments using purified immune cells fail to model the various interactions of immune cells with other cells and substances in the body. Moreover, such tissues contain complex mixtures of both immune and non-immune cell types each of which can signal to lymphocytes and affect their behaviour. Insights into whether specific components of the immune system can be targeted to improve therapeutic outcomes are also difficult to gain using experiments performed *in vitro*.

A retrospective assessment of replacement will be due by 20 October 2028

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 provided the estimated numbers of mice to be used under each protocol of the license. Use of littermate controls is a requirement in our experiments to ensure an identical environmental and genetic background between experimental and control animals in experiments. We calculated the number of mice required per line to produce sufficient experimental and control animals from heterozygous (consisting of different alleles of particular genes) crosses to allow for experiments to be conducted with adequate statistical power. The calculated numbers are sufficient for the generation of genotyped control and experimental animals at a typical age (range 8-12 weeks) at which they can be used in experiments. Our calculations are based on average litter sizes of 6, an average time between litters of 6 weeks, an expected sex ratio of 0.5 and an expected Mendelian ratio of 0.25 for experimental and control progeny.

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

We monitor the literature for methods to reduce the numbers of animals required and to improve experimental design. If a suitable allele has been generated elsewhere, we will import this by embryo or sperm transfer, or by shipping live mice where sperm or embryos are unavailable, and inter-crossing the offspring to obtain the required genotype. Cryopreservation and re-derivation by embryo transfer are important for both the quality of the science and for reduction in the numbers of animals bred.

Genome editing technologies such as CRISPR/Cas9 mediated gene editing offer a potential for reduction, since they allow the function of genes to be tested without germline mutagenesis and establishment of mouse colonies to test gene function. We have started to develop powerful new high- throughput CRISPR/Cas9 screening approaches to identify functionally relevant genes within specific immune cell types *in vitro*. While not relevant to a substantial proportion of our present research, this approach has the potential to lessen our need to perform preliminary testing of gene function using germline mutagenesis with subsequent reduction in mouse usage.

Experimental control groups are important for most of the experimental models being used and provide a means to improve data quality from our experiments involving animals. The generation of high quality data eventually provides a means of reduction since definitive results will prevent the necessity to perform experiments again, either due to technical failure or a lack of broad applicability/interpretability to other researchers in the field. Where relevant, we will use resources available for designing experiments with an appropriate randomisation and blinding strategy and sufficient sample sizes to enable adequate statistical power, such as the NC3rs experimental design assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda).

The use of IVIS live imaging will enable reduction of mouse usage by providing information on cell migration and immune response kinetics that cannot otherwise be obtained without euthanasia of animals and analysis of tissues at serial timepoints, providing a means for reduction of mouse 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 monitor the breeding performance of our mice to ensure that the minimum numbers of animals are used. We use colony management software that helps avoid overproduction. The use of inbred strains minimizes variation and allows us to robustly ascribe the cause of phenotypes observed to the introduced genetic mutation. Our research will require the generation and maintenance of complex multiallelic mouse genetic strains at colony sizes sufficient to provide littermate experimental and control animals from heterozygous matings. The number of 37000 reflects the number of mice required both at the crossing stages when different alleles are being combined onto the same background, and at the colony expansion and maintenance stages. Our calculations were based on previous experience generating and maintaining multiallelic mouse strains.

The maintenance of specific pathogen-free health status and controlled environment will reduce experimental variability and decrease the cohort size required for sufficiently powered statistical analysis. Staff receive training in biostatistics. Biological plausibility, mechanistic insight and consequences of the effect will inform the interpretation of significance in addition to obtained statistical significance values. Studies will be reported according to ARRIVE guidelines.

A retrospective assessment of reduction will be due by 20 October 2028

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 general, suffering will be minimised by careful observation of the mice undergoing procedures and adherence to AWERB handbook guidelines for the assessment of clinical signs that will trigger the end of an experiment for a particular mouse or a cohort. Detailed written guidelines have been recently produced for the care of aged mice. Where an option of experimental models are available to answer a specific question, we will chose the model which causes the least suffering to animals.

Ageing mice: we prioritize using mice that have become singly housed, as soon as possible. In consultation with animal technicians and veterinary staff, we modify protocols and introduce refinements to the protocols and animal husbandry that reduce harm. This includes alternative bedding for animals with reduced mobility, providing access to food in gel format, use of analgesics and more frequent monitoring for mice at increased risk.

Choice of experimental models: For example, where tumour immune responses are being tested, we will primarily implant tumours by subcutaneous injection, also called the

heterotopic tumour implantation model, and this model minimises animal suffering by enabling non-surgical introduction by needle injection of tumour cells into animals. Further, the subcutaneous space provides a compliant space for tumour growth resulting in minimal suffering to mice as tumours develop. It will also administer analgesia. In general, heterotopic models are preferable from an animal welfare perspective than orthotopic models (surgically implanting cells in recipient animals) and therefore heterotopic models form the mainstay of the proposed work involving tumour immunity although orthotopic models in compliant and non-surgically implantable locations will also be used (eg melanoma tumours injected into the intradermal space, and breast carcinoma cells injected into the mammary fat pad).

Virus or bacterial infection: Infection with influenza virus or live bacteria will be carefully controlled to minimise adverse effects. Batches of infectious agents will be standardised using in vitro assays and the lowest dose sufficient to elicit the required immune response given. Additionally, we have established clear humane end points for infected animals to avoid unnecessary culling of infected animals in experimental groups.

Perioperative and post-operative analgesia: Perioperative and post-operative analgesia will be given when necessary using advice from the NACWO and NVS. Choice of analgesic, duration and dose will be adjusted to the clinical signs observed taking into account possible impacts on the experimental plan. Peri-operative analgesia will be given and maintained after surgery for as long as is necessary to alleviate pain. Analgesia will also be administered to control pain for painful procedures. In addition, procedures are refined to reduce the suffering that they cause and training of staff conducting procedures is maintained so as to minimise inter-operator variation and suffering.

Administration of tamoxifen: Administration of tamoxifen in the diet is known to cause neophobic effects in mice. We have gained experience and information from our project support team that has enabled us to make refinements to our tamoxifen administration protocol reducing adverse events related to tamoxifen-induced neophobia substantially.

Experimental autoimmune encephalomyelitis (EAE): EAE has a severe severity limit and is likely to cause substantial clinical signs in about 10-20% of the animals. It is necessary to use this experimental system because there are no other ways of modelling the relevant neuro-immune interactions underlying multiple sclerosis using less severe protocols or in vitro methods. To minimize animal suffering, we will carefully monitor the animals' condition, provide access to food and water in the case of immobility, and euthanize animals prior to exceeding severity thresholds. For example, we will provide DietGel and heat-pad to animals in the case of paralysis and/or weight loss. This model is widely recognised as one which most reliably allows measurement of autoimmune T cell-mediated tissue injury in the CNS. There is a great unmet clinical need in this area and our experiments could inform the development of new therapeutics.

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

Lesser model organisms, such as zebrafish, have far less conserved immune systems to humans than mice, are less well characterised and many of the tools used to study immune function in mice have not been generated for lesser species. Thus, while we have considered other ways to reduce and refine our use of animal models, our funded programme of research would be impossible to complete using less sentient species than mice. Access to primary and secondary lymphoid organs and peripheral tissues, such as the lungs, gut, spleen and lymph nodes, is required for the proposed analyses and such access is limited in human studies. Adoptive transfer experiments are also possible using

inbred mouse strains since they are genetically identical and do not express mismatched antigens for graft rejection.

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

We constantly refine our experimental techniques to prevent unexpected adverse events, minimise variability and thereby reduce experimental group sizes, and minimise suffering caused to animals by working with small groups of animal technicians longitudinally on specific projects so that experience of experimental techniques builds over time, and by producing and refining standard operating procedures for interventions such as cell injection and tumour measurement.

Where it is necessary, we will monitor animals daily, such as by measuring weight and observing behaviour. We will keep close contact with animal technicians and kill animals once any significant clinical signs show.

To reduce the pain caused by the administration of any immunoregulatory substances, we will seek advice from animal technicians, NACWOs and NVSs.

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

We will follow these guidelines below:

Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy.

Lab Animal 46(4):152-156. doi:10.1038/laban.1217

LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.).

Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T (2018)

PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines

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?

We will have regular discussions with the researchers and animal facility technicians and managers at our institution to review current approaches and whether there are any new 3Rs.

Research staff will subscribe to the NC3Rs e-newsletter, providing updates on updates to 3Rs principles and methods, funding opportunities, 3Rs events and publications.

We will encourage staff to attend NC3Rs events and workshops as a way of keeping abreast of 3Rs advances and approaches.

We will keep in touch with NVSs (Named Veterinary Surgeons) and NACWOs (Named Animal Care and Welfare Officers) and animal technicians for seeking advices when necessary.

A retrospective assessment of refinement will be due by 20 October 2028

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?

11. Developing Genetic Approaches to Cancer Treatment

Project duration

5 years 0 months

Project purpose

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

Key words

cancer, targeted treatment, drug resistance

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.

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?

Although treatment is effective for many people diagnosed with cancer, a significant proportion of the cancer patient population either do not respond to treatment (de novo resistance) or become resistant to treatment over time (acquired resistance). In most of these cases, the cause of therapy resistance is not known and approaches to limit its impact undiscovered. Furthermore, whilst targeted treatment approaches are available for many cancer patients, this is not universal. This project aims to use animal modelling of cancer to: (i) understand how treatment resistance in cancer occurs and how it might be targeted; and (ii) identify targeted approaches to treating cancers of the breast and ovary.

A retrospective assessment of these aims will be due by 13 December 2028

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?

Failure to identify refined ways of treating cancer will result in higher rates of cancer-related mortality.

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

This work will generate new information and data that will be used to inform the design of new drug discovery and drug development projects and the identity of new criteria that could be used to determine which patients will benefit from which drugs, based on the genetic changes in their tumours.

We hope to discover new treatment options for patients with cancer based on information about changes that are found to occur in their tumour cells. One way will be through using mouse models designed as part of the EU Infrafrontier model-making effort to study how cancers stop responding to treatment.

We also have mouse tumour cell lines and cell lines derived from patient tumours grown in mice (patient-derived xenografts) that can acquire changes that stop drug response. We will use these

models to:

- Test and analyse results for drugs that we have identified and propose could reduce cancers returning especially after initial response.

- In some mouse tumours and cell lines, we will test and analyse whether alternative approaches could be beneficial in treatment.

We will also use appropriate tumour models to confirm findings from our in vitro studies, as a step towards establishing clinical trials for new drug indications. This is especially important in cancers that still lack optimal treatment options such as ovarian cancer and some types of breast cancer (lobular and triple negative breast cancer).

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

Short term beneficiaries will include other scientists and oncologists working in this area, who will benefit from the information generated by the work. As we aim to use the information gained in the project to design new drug discovery and drug development projects, the long-term beneficiaries will be people diagnosed with cancer.

We work closely with patients who are likely to benefit from milestone in the progression to testing drugs and novel therapeutic approaches as well as allowing us to get an early understanding how drug resistance might emerge.

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

We will disseminate the information gained from this project via publication in peer reviewed journals and the presentation of scientific presentations at national and international conferences.

Internal and external collaborations will also benefit from material and data from this work.

Species and numbers of animals expected to be used

- Mice: 14200

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 proposal is to address challenges in cancer treatment with well-established mouse models of cancer. There are advanced technologies to genetically engineer mice available, making mouse tumours a good system to study how genetic changes in cancer can lead to treatment sensitivity or resistance.

Many of the proposed experiments require an immune system and are therefore best studied in an animal model such as a mouse where this is the most faithfully recapitulated. We will also take complementary laboratory approaches (for example using cell cultures and computational analysis of data from patients) to minimise animal use whereever possible

In experiments where human tumours are grown in mice (xenografts), we will make use of genetically engineered mice that lack a functional immune system. This means that tumour cells from a foreign species (human) can be transplanted without triggering an immune reaction.

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

A typical scenario:

An animal will receive an identification mark (mild).

Health checked and weighed to certify as suitable to start study.

The animal will be implanted with tumour (moderate) and monitored gentle palpation or calliper measurements.

When tumour is established, animal receive treatment once a day (moderate) for a previously considered optimal duration for the drug.

Animal may continue to be monitored for tumour size change when treatment stops. At the end of the study, animals will be culled by a schedule 1 method.
What are the expected impacts and/or adverse effects for the animals during your project?

Majority of the animals have tumour growth. Half of the tumours developing on this project will be superficial (close to the skin unlike deeper tissues such as lung and liver cancers). These superficial models rarely have an effect on normal activities including acquisition of food, water and movement.

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 on this project licence. mild-5000 (35%) for breeding. moderate-9200 (64.8%) such as injecting cancer cells followed by treatment. severe-200 (<0.2%) small pilots to check if new drugs will be safe.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 13 December 2028

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?

Many aspects of tumour biology and drug toxicity cannot be fully studied in isolated cell or biochemical models. Using animal systems provides a complement to other laboratory studies in which the whole organism context can be understood. In particular, the interface of tumours and cancer therapies with the immune system is extremely difficult to model accurately without using animals.

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

Majority of the work is in cancer cell lines cultured in flasks and tumour organoids (cells from patient tumours grown in the lab) and have used computational analysis. Such systems allow an alternative to growing tumours that does not involve transplantation into mice, and thus reduces the number of mouse experiments required.

Why were they not suitable?

These systems are used as a first step in research to ensure that only promising hypotheses are taken forward into animal models. Although these can replace animal

models from the point of view of looking at the tumour cells, they generally do not allow studies of the normal cells. This means, for example, the effects of candidate drug treatments on normal tissues cannot be studied, nor the effects of immune responses on cancer treatment.

A retrospective assessment of replacement will be due by 13 December 2028

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?

Animal numbers are based on prior experiences with the model system in question, and best estimates of expected effect sizes. Where appropriate we carry out pilot studies to establish these parameters and allow us to accurately determine the appropriate animal number for larger experiments.

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

We carry out as much work in vitro as possible using surrogate models (such as cell lines and patient derived organoid models) in order to ensure that only the strongest hypotheses are progressed to in vivo testing. We use statistical tools such as those on the N3CRs website, in combination with estimates of effect sizes to ensure that experiments are of an appropriate and not excessive size.

We have made extensive use of computational analysis of data from experiments and patients to come up with the most confident predictions possible about which genes will affect cancer drug responses. Only the most promising candidates with supporting evidence from laboratory experiments will be taken forward to testing in animal models. This ensures we only use animal models where appropriate and where there is a good likelihood of success.

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 where appropriate to determine parameters such as tumour graft success rate for each model before proceeding to larger experiments. Standard mouse strains are purchased from commercial breeders rather than bred in house as this is more efficient and reduces the number of colonies maintained.

Tissues and tumour passage material is carefully labelled and banked, documented systematically and can be shared internally and with collaborators.

A retrospective assessment of reduction will be due by 13 December 2028

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 widely used for preclinical models for cancer research, with the benefit of vast amounts of published data to support our research and decision making. The animals are maintained in ventilated cages using sterile food and bedding and all procedures are carried out in cabinets with special air handling to avoid infections. We use cage enrichment to improve the living environment for the mice.

Majority of our animals will experience moderate severity, we are constantly looking for ways to improve the animal experience through training, meeting weekly with collaborators. For example, one of our surgical techniques has been refined to be completed in 20 minutes from 40 minutes.

The institute now has an ultrasound machine and In vivo persons on the team have received training for guided injections to minimise tissue damage.

Animal suffering will be minimised by daily checks to ensure that any problem is noted as quicky as possible, tumour burden will be kept within acceptable limits. Therapeutic drugs will have been assessed for toxicity and therefore we expect high tolerability of the regimes.

We use analgesia as part of all surgical procedures, thus minimising suffering. We constantly refine our protocols to account for current best practices including contemporary analgesia regimes, learning refined surgery skills through collaborations and training.

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

Cancer, in particular breast cancer, is best modelled in mammals where the relevant organs, immune system and other physiology are similar to humans. Using less sentient species would risk the research not being relevant. Due to the typical time course of tumour growth (generally several weeks or months), using immature or anaesthetised animals is not possible.

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

We have weekly meetings with other teams carrying out related projects. This way we are able share challenges and opportunities to apply improvements of techniques, animal care and pain management during the duration of the project.

Where animals seem unwell unexpectedly, the Named Veterinary Surgeon will be contacted for advice.

Our in vivo team personnel have regularly received training from accredited organisations and this will continue during the duration of this project.

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 by Workman et al, 2012

The NC3R website offers up to date guidance of experimental design including the Animal Reporting of In vivo Experiments (ARRIVE) guidelines which we apply to each of our study designs prior to PPL Holder approval.

Principles of aseptic surgery are widely published on different organ systems.

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

Through regular scheduled meetings with the named persons of the establishment (NTCO and HOLC) and discussing how to apply new information received concerning animal work.

In vivo members of the team are also registered with the NC3Rs information list and hear about seminars and trainings that are available throughout the year to apply improvements.

Funding has been allocated to continue training such as pain and refinement to the in vivo persons on the team with accredited Institutions.

A retrospective assessment of refinement will be due by 13 December 2028

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?

12. Understanding and Improving Central Nervous System White Matter Disease

Project duration

5 years 0 months

Project purpose

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

oligodendrocyte, myelin, white matter, multiple sclerosis, small vessel disease

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 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?

To better understand and improve white matter neurodegenerative pathology in the central nervous system.

A retrospective assessment of these aims will be due by 30 November 2028

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?

Many neurodegenerative diseases of the brain and spinal cord affect humans and most are incurable. Increasingly, oligodendrocytes, which make myelin, are recognised as involved in many, and as these are renewable and manipulable, they are a target for better therapeutics for diseases as diverse as multiple sclerosis, dementias and Huntington's disease. We believe that if we can understand these cells better, their relationship to other brain cells and their response to disease, we will be able to discover better ways of modulating these diseases for benefit in humans.

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

We expect to gain:

- A greater understanding of the biology of glial cells and their interaction with other cells of the central nervous system, and how this changes in disease.

- New strategies to manipulate this biology/pathology to ameliorate neurodegenerative disease.

- New potential molecular/pathway targets as potentials for future therapeutics for neurodegenerative disease.

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

Scientific community - increased knowledge about glial biology in neurodegenerative diseases in short and longer term. Training of junior scientists in these approaches (short-medium term).

Translational scientific and Pharmaceutical communities - generation of new therapeutic approaches and potential therapeutic targets in medium and longer term.

Patients/general public - increased understanding about neurodegenerative diseases in short term and with longer term translation, therapeutic benefits.

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

We will publish datasets of all of our studies regardless of outcomes. We will also maximise outcomes of our research through collaborations as well as presentations in national and international conferences. We are very engaged in public outreach work to disseminate our findings to the general public.

Species and numbers of animals expected to be used

- Mice: 6000
- Rats: 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.

We will use rodents for this research, which are suited for studying neurodegenerative diseases. For demyelinating neurodegenerative diseases (such as modelling multiple sclerosis), we will mostly use mice, as mice models show changes similar to those seen in these human diseases and we have genetically modified mice that allow us to better model these or track individual cells helping us understand the diseases better. Rats will be used more for studying vascular dementia (cerebral small vessel disease) as they have more complex behaviours (related to more white matter in brain) allowing improved relevance of cognitive testing.

We will assess rodents at all developmental stages including prenatal stages of development, adulthood and old age as vulnerability to these diseases can be genetic, (hence present at birth) and pathology generally worsens with ageing.

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

Animals used in this project will undergo surgery, injections of substances, brain implants, testing for nerve activity, imaging under anaesthesia, culling under anaesthesia and behavioural assessments.

Animals may also be identified with tattooing as well as radio-frequency identification microchipping to ensure that identification can be reliably performed without fail.

Experimental durations will range from acute (1 day) to several months, to many months (in ageing studies).

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

Potential adverse effects include possible death under anaesthesia in survival surgeries, pain following surgical procedures which will be significantly reduced with analgesia. Global demyelination can lead to weight loss (which will be monitored). Modelling vascular dementia can cause behavioural change, but this is subtle and does not alter feeding 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)?

- Mice:

- Mild - 30%
- Moderate - 50%
- Severe - 20%
- Rats:
- Mild - 30%
- Moderate - 60%
- Severe - 10%

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

- Killed

A retrospective assessment of these predicted harms will be due by 30 November 2028

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?

In order to understand how the brain and spinal cord are altered in neurodegenerative disease, we must use technically advanced methods modelling these diseases in whole organisms including imaging, transgenic and pharmacological manipulations of the mammalian central nervous system. We can (and do) study some of these processes where possible in dishes (in vitro) and with human cells but these are limited and ultimately we must involve the use of vertebrate animals to faithfully reflect the complexity of disease.

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

Human cell and tissue preparations.

Why were they not suitable?

Human cells grown in vitro and biopsy/post mortem tissue is vital to our research, and we use these a lot. However, in vitro cell experiments cannot model the complexities of a human brain or its interaction with the rest of the body, and human tissue provides, by its nature, a single sample at a single time- point. Therefore, it is essential to complement this work with rodent in vivo work.

A retrospective assessment of replacement will be due by 30 November 2028

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 estimate the given numbers of experimental animals based on usage statistics of previous publications as well as information provided from other license holders at our establishment. The numbers required for upkeep and desired out-breeding of lines are derived from estimates provided by the animal facility. To stay updated on optimized experimental design, we will regularly seek guidance from biostatistical experts employed by our establishments.

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

We use fewer live animals by using cells (including human cells) or brain slices grown in a dish for many initial experiments. We carry out pilot studies where possible before planning for large cohorts. We calculate the correct number of animals required for an experiment (using the NC3R Experimental Design Assistant) to show an effect using statistics, so we use enough but not too many, in accordance with the ARRIVE2 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 will implement efficient breeding procedures and will additionally perform well-designed pilot studies whenever possible to assess the potential for publishable results. We will additionally perform computational modelling and analysis of datasets to maximize the information extracted from our datasets.

A retrospective assessment of reduction will be due by 30 November 2028

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 rodents as animal models to better understand how the cells of the brain work and how they respond to neurodegenerative diseases - especially focussing on multiple sclerosis, cerebral small vessel disease (dementia) and Huntington's disease.

We will use wild-type and transgenic rodents which in the unperturbed state will experience no harmful effects. Some transgenic models develop dementia-like conditions over time, but are able to feed and move normally. Some animals will be given pathologies either using substances or surgery that increase neurodegeneration in a similar way to the human diseases, so that we can try and improve these using therapies.

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

Neurodegenerative diseases alter the complexities of the human brain and generally worsen with ageing, and so cannot be studied in less sentient animals with different nervous systems.

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

Any of the studies involving surgical or invasive intervention will adopt appropriate pain management and post-operative care.

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

We will stay up to date on best practice guidelines from the NC3Rs website (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?

We will stay informed about the advances in the 3Rs by attending informational events provided locally and provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

A retrospective assessment of refinement will be due by 30 November 2028

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?

13. Cellular and Molecular Mechanisms Underlying the Pathogenesis and Remission of Arthritis

Project duration

5 years 0 months

Project purpose

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

Key words

arthritis, inflammation, disease remission, myeloid cells, immune-regulation

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 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?

- We will investigate the biological reasons why people get arthritis by identifying the molecular mechanisms in cells that contribute to disease.

- We will identify faulty mechanisms in cells of patients with arthritis and this new knowledge can help design new medicines to improve treatments.

A retrospective assessment of these aims will be due by 15 December 2028

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?

- Current medicines for arthritis don't prevent recurrence of symptoms and new medicines are needed.

- Many arthritis patients do not tolerate or respond to current medicines, and the improvement is typically temporary in those that respond. Some medicines have powerful side-effects but reducing the dose can cause recurrence of arthritis. Patients with arthritis are prone to develop lung and skin disease. Arthritis has many causal mechanisms, and new understanding of this diversity as outlined in this licence is required to design better medicines.

- Our preliminary studies identified molecular complexity of cells in patients with arthritis that need to be validate by protocols outlined in this licence.

- New knowledge will match patients to appropriate medicines. In patients who don't respond to current medicines, new disease mechanisms will be identified to help design new medicines that might control disease.

- Extending the stability of the treatment response, with the prospect of cure.

- We have strategies to re-educate cells of the immune system to resolve arthritis. Reprogramming these cells with new medicines might then permanently prevent further joint damage, offering the prospect of cure.

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

New knowledge: This project will generate new information of molecular mechanisms to design new medicines that:

treat arthritis that does not respond to current treatment

sustain long-term disease remission

re-educate immune system to reverse the aberrant response to joint tissue

This new knowledge may be also applicable to lung and skin conditions associated with arthritis and will help in the understanding of other abnormal inflammatory diseases.

Products: New strains of mice will be generated to test the scientific aims. They can be shared with other groups upon request to facilitate discoveries in other diseases.

Publications: At least 5 high-impact open-access publications describing new mechanisms of disease resolution, and potential medicines that re-instate healthy joint. Relevant data will be made rapidly available in the form of pre-prints in repository journals and on social media.

For patients and clinicians: In long term, the work on this licence will deliver medicines that more appropriately target arthritis, prevent flare of disease and minimise side-effects of inappropriate treatments.

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

Overall benefits: This project will provide new knowledge of the identity and function of the cells and molecular mechanisms that are responsible for the driving and resolving joint inflammation. This will benefit arthritis patients, clinical rheumatologists, pharmaceutical industries, and academics interested in mechanisms of immune regulation and autoimmune diseases.

Arthritis patients: Remission of arthritis is defined by patients as “being able to do normal activities”. Our new insights into mechanism of treatment-resistant arthritis will give encouragement for patients that are in despair after frequent treatment failures and restore confidence that we are responding to patient needs.

Clinical rheumatologists: Some cells and molecules identified in this project might be indicators (biomarkers) of treatment response, thus could be used in the clinic to predict whether a patient will respond to a particular treatment.

Pharmaceutical industries: new knowledge from our current and ongoing discoveries from this project will provide an evidence-base of therapeutic targets that will help Pharma design new treatments for arthritis.. Some companies have shown considerable interest and responded to our pilot findings and have provided funding to support work in this licence application.

Academic Rheumatology and Immunology: Findings will be made available by publication in peer- reviewed high-impact journals and presentations at scientific conferences and meetings.

Basic science: This work will advance fundamental scientific knowledge of how immune cells contribute to tissue pathology and repair that is highly relevant to all chronic disease.

Charities that support arthritis research: will benefit from our discoveries that directly address patient needs by providing clear evidence of progress. This feedback to donors will encourage their continued support.

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

Collaboration: We have ongoing collaboration with other UK and EU specialist centres that will help us to use the best technologies to address our objectives.

Dissemination: Our work will be disseminated annually at appropriate national and international medical and scientific conferences including British Society of Immunology (BSI), European Immunology conference and European and American Rheumatology Conferences.

Publication of unsuccessful approaches: Along with data describing new mechanisms driving pathology, maintaining disease remission and restoration of healthy tissue, we will publish all experimental evidence including negative results to provide a broader knowledgebase for the biomedical scientific community. This would include sending our manuscripts to repository journals e.g. bioRxiv.

Species and numbers of animals expected to be used

- Mice: 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 will use adult mice. The immune system of mice is well-studied and sufficiently similar to that of humans to be of great value. There are many treatments validated for use in mice that we will use to investigate arthritis. There are several mouse strains with specifically altered genes that will enable us to test our research questions including discovery of mechanisms that lead to the resolution of arthritis.

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

There are several well-described and validated by scientific community experimental (murine) models of arthritis. These include injections of biological molecules such as antibodies, proteins such as collagen or cells systemically or locally into the joints. The duration of each model is typically 2 months.

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

Generating arthritis in mice can lead to prolonged pain when disease becomes severe (5-10% of animals). If pain-killer medicine is used, the same painkillers will be given to matched-control mice in order to standardize the experimental protocol. Mice are monitored daily, and those that lose more than 20% of weight (from start of the experiment) and those that have footpad swelling exceeding 4.5mm will be euthanised 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 severity of arthritis and its systemic complications is ‘Moderate’. However, based on our experience (from previous two licences) 5-10% of mice can develop severe disease.

Minimising animal suffering: Routine daily monitoring measures are in place to reduce suffering. There is a Welfare Scoring System for mouse arthritis. Mice developing joint swelling or skin and lung inflammation will be monitored for signs of malaise, e.g., listlessness, hunching, weight loss. Food (including a soft diet option) and water will be placed within reach on the floor of the cage. Extra bedding and nesting material will be added for comfort. Weight will be monitored daily.
At the peak of the inflammatory response animals might show signs of pain such as rapid, shallow breathing, grunting on expiration, and loss of limb movement (arthritis). Pain-killer medicine might be given to these mice in consultation with the duty veterinary surgeon.

Mice will be humanely euthanized with evidence of either:

footpad swelling exceeds 4.5mm (arthritis)

weight-loss becomes ≥20% of the weight preceding procedure.

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

- Killed

A retrospective assessment of these predicted harms will be due by 15 December 2028

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 identified previously unrecognised complexity in the molecules and cells causing arthritis using cells from patients' joint biopsies. This complexity is responsible for the fact that patients don't respond well to current medicines. The animal models will help us to discover how these cells and molecules interact to cause arthritis. It will help to discover potential new medicines that later could be tested in humans.

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

Our project predominantly uses blood and joint cells from arthritis patients obtained ethically as part of their routine assessment. The function of these cells can be tested in the laboratory to reduce the need to use animal models. For example, we developed precision methods to obtain new information from cells in a laboratory dish, that will guide highly specific mouse experiments for maximum benefit.

We demonstrated that when some blood and joint cells are mixed in a laboratory dish they can spontaneously assemble into mini structures (called organoids) that are similar to structures in the human joint. We created organoids similar to healthy joints and arthritis joints and these were useful alternatives for experiments that substantially reduced the need for mouse models. However, the discoveries using these laboratory alternatives require testing in live mouse models.

We will remain vigilant for alternatives using the following resources:

The EURL ECVAM Search Guide on alternative strategies and methods to animal-based research.

FRAME: a resource on the basic principles of searching for 3Rs information.

NORECOPA: a series of databases, including on alternatives to the use of animals in education and training.

Why were they not suitable?

The complexity of cell interaction causing human arthritis that includes skin and lung disease cannot be adequately reproduced in the laboratory using cells from patients. The natural interactions of these cells require using a live model of arthritis that is best done in mice.

In addition, testing if drugs are affective for arthritis is more convincing if done in live mouse models than testing individual cells in the petri dish.

A retrospective assessment of replacement will be due by 15 December 2028

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?

For the experimental protocol (protocol 2) included in this licence application, 20 mice to test one scientific question will be sufficient. These include 10 mice per experimental group (treated or transgenic) and 10 mice per control group (untreated or wild type)

Biomedical journals require that animal experimentation should be repeated 3 times, therefore 60 mice per one scientific question will be required.

In summary, we estimate that we will use 1000 mice per year for experimental protocol 2 (5000 in total) and 2000 for breeding in protocol 1.

Total number 7000.

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

We will continue to use the Experimental Design Assistant (EDA) to guide experimental minimise and optimise mouse numbers by

- accounting for the influence of variables and addressing sources of bias which will optimally design the experiment to yield robust and reproducible data, and to ensure that the data from each mouse is utilised to its full potential.

- an efficient use of statistics to reduce the number of animals required and maximise the information obtained per 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 will minimise the number of mice kept in reserve and will, as far as possible, breed on demand.

- We will use both males and females in experimental prototcol.

- A research-group member identified by the project licence holder will deputise as the manager for each colony to monitor and report when sufficient animals are available to ensure high quality science, while minimising avoidable production of surplus animals.

- We will plan to optimise the number of control mice for all experiments including a system for making control mice available between different experiments

Pilot studies: Every new experimental hypothesis will be tested in pilot studies using small numbers of mice (n=3) and high-resolution analysis to assess whether subsequent experiments are justified.

Computer modelling: We are developing Machine Learning tools (Explainable Artificial Intelligence) to analyse how cells from patients with arthritis can cause disease and how they differ from healthy subjects. The dominant cell functions causing arthritis will be identified and drugs that can potentially prevent these functions will be prioritised for testing in the mouse model of arthritis. This will reduce the number of mice required to test and validate the discoveries using human cells.

Optimising the use of mouse tissue. All three tissue type under study (joint synovium, lung and skin) from each mouse undergoing schedule 1 can be harvested and studied, or cryo-preserved to serve as control tissue for pilot studies and/or as training material.

A retrospective assessment of reduction will be due by 15 December 2028

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.

Choice of models: Mice don’t normally develop either rheumatoid arthritis or arthritis-associated lung and skin diseases. Arthritis models therefore require stimulation, or disruption of cells or molecules involved in inflammation. Below are the models proposed in this application. These models are accepted by both the scientific community and the Home Office as representative of human arthritis.

Arthritis models called (i) collagen induced- arthritis, (ii) breach of tolerance arthritis, and (iii) antibody- induced arthritis are well recognized models, and each model allows investigating different stages of the human disease. To replicate inflammation in lungs and skin that are co-complications of human arthritis, these models can be modulated by intra-nasal or intradermal administration of appropriate substances such as type 2 collagen or bleomycin that are accepted to induce experimental skin and

lung inflammation. These models are necessary to investigate the therapeutic potential of our discoveries made in cells from arthritis patients in treating inflammation in joints, lung and skin.

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

Mice are the least sentient animals that have a well-described immune system that is similar to humans. They provide the optimum species to test and validate the therapeutic potential of our discoveries in human synovial joint cells.

Arthritis and its complications develop predominantly with age - typically older than 40 years, thus we will model arthritis in adult mice.

Pathogenic and disease-resolving mechanisms of arthritis are complex and involve dynamic and prolonged interactions between different cells and molecules and therefore need to be model on live mice and cannot be modelled in terminally anaesthetised mice.

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

Minimising animal suffering: Mice on experimental protocols will be monitored daily and those that lose ≥ 20% of weight or have severe arthritis (usually 5-10% of animals) will be euthanised. In addition, extra bedding, soft food, increasing ambient temperature and application of analgesia will be considered. We will consider opioid based analgesia which was reported to have no impact on the immune-response/inflammation while reducing pain in wild type animals (DOI 10.1007/s10787-015- 0241-4). Since there is no data on the effect of opioid based analgesia on immune reponse/inflammation in the GA listed in our licence, if appropriate (e.g. an increase in % of animals reaching severe disease in GA lines) we will design the pilot study to test the effect of opioid on immune response in these mice.

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

- We will follow guidelines of the local Animal Welfare and Ethical Review Body to ensure that all use of animals is carefully considered and justified; that proper account is taken of all possibilities for reduction, refinement and replacement (the 3Rs); and that highest standards of accommodation and care are achieved.

- We will follow ARRIVE (Animal Research Reporting In Vivo Experiments guidelines - 20-point check list (Plos Biology June 2020) to ensure that the data from animal experiments can be fully evaluated and utilised.

- We will regularly screen the NC3Rs gateway for current 3Rs advice.

- We will incorporate recommendation on caring for animals on arthritis protocol from "Applying refinement to the use of mice and rats in rheumatoid arthritis research" DOI 10.1007/s10787-015- 0241-4

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

We are engaged with the 3R aims by predominantly working with patient-derived cells and human organoids. We also use Explainable AI to prioritise molecules for testing in animals with the highest potential of therapeutic benefit for patients.

We engage with the NC3Rs (National Centre for the Replacement Refinement & Reduction of Animals in Research) to be continuously informed about advances in 3Rs, and an assigned research group member will report on this as an agenda item to monthly meetings.

We actively review the updated 3Rs approaches on the NC3Rs gateway (hosted by F1000Research) that publishes novel 3R models and technologies, and describes the methodological and technical details sufficiently to be reproduced. This publication also includes a realistic evaluation of the scientific, 3Rs and practical benefits of the approaches described, along with their current and potential applications, and details of their validation against current “gold standard” models.

A retrospective assessment of refinement will be due by 15 December 2028

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?

14. Investigating the Role of Platelets in Thrombosis and Inflammation during Sepsis

Project duration

5 years 0 months

Project purpose

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

Key words

Sepsis, Inflammation, Platelet, Immune cells, thrombosis

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 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?

Platelets are small blood cell fragments essential to stop bleeding at the site of injury. They also contribute to inflammation and clotting during infection, a pathological condition which can progress to sepsis. The aim of this project is to understand the role of platelets in inflammation and clotting under these pathogenic conditions. As inflammation and clotting occur in different organs, we aim to understand the contribution of platelets to inflammation and thrombosis in different organs and to identify potential targets to limit their detrimental effect. Finally, we will assess the efficacy of drugs targeting the pathways identified in improving organ function and reducing clotting associated with sepsis.

A retrospective assessment of these aims will be due by 10 November 2028

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?

Sepsis leads to life-threatening, severe ly dysregulated inflammatory response associated with multiple organ damage, which affects over 19 million patients worldwide annually. In the UK, sepsis is responsible for 40.000 deaths per year. During bacterial infection-mediated sepsis, immune cells are essential to clear pathogens. However, uncontrolled immune cell activation leads to excessive inflammation and thrombosis (clotting) which can damage different organs. Inflammation and thrombosis can result from the pathogen itself and/or from the host response, even once the pathogen is cleared. Moreover, one of the challenges in treating sepsis-associated thrombosis is the concomitant risk of clotting and bleeding. Therapeutic strategies targeting inflammation and clotting during sepsis must preserve the beneficial role of the inflammatory reaction in clearing bacteria and reduce clotting without increasing the risk of bleeding. Another major challenge in targeting thrombosis during sepsis is the heterogenous composition of the clot and the development of distinct mechanisms in different organs. Therefore, understanding how inflammation and clotting develop and progress in each organ and how platelets contribute to these events is essential to reduce clotting and organ damage during sepsis.

Currently, there is no successful treatment for sepsis. Infection has many microbiological factors, timescales and deferentially affect organs, along with patient genetic and environmental factors and comorbidities which can each influence sepsis outcome. So far, most studies assessed the efficacy of classical anti-thrombotic drugs during sepsis. These drugs failed to improve outcome and increased the bleeding risk. Here, we propose to assess the role of novel proteins which, until recently, were not known to regulate clotting. Importantly, these proteins are not involved in classical haemostasis (to arrest bleeding at the site of injury), giving a unique opportunity to explore the potential of targeting these novel pathways in sepsis, without taking the risk of increasing bleeding risk. This work allows a better understanding of the mechanisms regulating clotting during sepsis, in particular the role of platelets, and to identify targets to reduce/resolve clotting without increasing bleeding .

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

The information generated will be published in peer reviewed scientific journals and presented at national and international conferences and can guide future studies and

possibly clinical trials for selective drugs in stratified patients based on their immune phenotype and the organ affected.

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

In the short term, colleagues and collaborators will benefit directly from the data generated as this will help to inform future experiments and project direction. Following publication of this data, other members of the research community will also benefit from the data being made available via open access.

In the medium term, depending on the findings and whether potential drug targets were identified, pharmaceutical companies might have interest in developing drugs targeting specific pathways or repurposing available drugs.

On the long term, based on the results, selective drugs can be proposed in small- or large-scale clinical trials in patients with sepsis.

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

The information generated through this work will be published in peer reviewed journal, presented at conferences, and further collaborations with other researchers and research groups will be established. Even in the absence of beneficial effect of anti-platelet drugs or immunomodulatory molecules tested in this project, data related to the mechanisms involved in thrombus formation in different organs during sepsis is needed to improve the knowledge of pathological conditions associated with sepsis. This work would allow to identify drugs with potential benefit and possibly rule out harmful drugs.

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.

I am interested in understanding the pathophysiology of clotting during sepsis occurring in adults, therefore I will use adult mice on this licence. Neonatal platelets have different reactivity compared to adult platelets and results cannot be translated to human sepsis in adults.

As a model of sepsis-induced inflammation, we will use lipopolysaccharide (LPS) to induce systemic acute inflammation. LPS acts through its receptor toll-like receptor 4 (TLR4), which is not expressed for example in zebrafish, limiting the use of less sentient species. Mice are being used in this project as an experimental model of sepsis, as the

development of the inflammatory response in different organs, the cells involved and targets are close to humans.

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

Typically, mice will be bred and undergo one of the two approaches to model two main pathologies in sepsis: inflammation and clotting. The first approach involves a single administration of the exogenous toxin lipopolysaccharide (LPS), the major component of the outer membrane of gram-negative bacteria. The animal is humanely killed after maximum 48 hours following LPS injection. This approach allows to investigate the role of platelet in thrombosis and inflammation in the absence of bacteria, which allows to assess host response in the absence of the pathogen, focusing on how host inflammatory response contributes to clotting. To target selective pathways, GA mice or pharmacological inhibitors will be used.

The second approach aims to assess the role of platelets in clotting in the context of live bacteria, using the animal model of sepsis, ceacal ligation and puncture (CLP). Mice will be humanely killed up to 48h following CLP. As we propose that inflammation is the main trigger of clotting during sepsis, targeting clots will improve organ perfusion and function. However, some clots act as a trap to capture bacteria and limit their spreading. Therefore, once identified a pathway that reduces clotting, we will validate that targeting this clot doesn't increase bacterial spreading. Therefore, the CLP bacterial model allows to assess this question to validate the efficacy of targeting specific pathways in limiting clots without increasing bacterial spreading. This procedure will be used as a secondary approach following identification of specific pathways using LPS model . To target selective pathways, GA mice or pharmacological inhibitors will be used.

In some cases, mice will undergo LPS or CLP and kept for shorter time for intravital imaging under terminal anaesthesia to visualise the early events occurring following induction of inflammation.

In some cases, in particular when GA mice or drugs were not previously characterized for their role in thrombosis, platelet function and thrombosis can be assessed under terminal procedure and this data will be used a control for sepsis models.

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

Both LPS and CLP induce an inflammatory response. LPS induces an acute inflammatory response whereas CLP induces a progressive inflammatory response. It is therefore expected to observe adverse effects indicative of an ongoing inflammatory response in the peritoneum, including piloerection, intermittent hunched posture, dehydration, reduced activity, and loss of appetite reflected in weight loss (maximum of 15% loss permitted in 48 hours).

To reduce these effects, mice will be routinely given supportive treatment (saline, analgesia , supplementary warmth). If these signs don't improve within 4h, mice will be humanely 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)?

The expected severities are:

- 90% moderate
- 10% severe (mice which are of unknown phenotype)

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

- Killed

A retrospective assessment of these predicted harms will be due by 10 November 2028

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?

Sepsis physiopathology involves a systemic inflammatory response that is not possible to replicate in vitro or ex vivo models. Therefore, the use of animals allows to investigate the causes of multiple organ damage and thrombosis and identify potential therapies. We are currently collaborating with colleagues to assess the possibility of developing and using a novel technology of organ-on-a-chip. We are generating organoids from induced pluripotent stem cells to generate organoids to mimic different organs. In the future, in case of success in generating these organoids and depending on their functionality, we will limit the use of mice as a model of sepsis and we aim to use the generated organoids to assess the damage induced by the infection.

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

A common cause of multi-organ failure during sepsis is microvascular thrombosis. The type of cells and organs affected during sepsis, from i) vessel wall, ii) immune cells and iii)

clotting, and the interplay between these cells in different organs can currently only be evaluated using animal models. We are currently assessing the use of newly generated organoids as an alternative approach; however, this is still at the experimental design stage. Moreover, we are assessing the possibility of using blood from septic patients perfused in artificial channels to mimic the blood flow, and we aim to use this model as a complementary approach to reduce and restrict animal use related to scientific questions that can be addressed in vitro and ex-vivo using human blood.

Why were they not suitable?

The models used in vitro can assess some but not all aspects related to sepsis. Sepsis leads to damage in multiple organs which cannot be mimicked using in vitro models. However, multi-organ failure is the cause of death in patients, and the crosstalk between different organs cannot be mimicked in vitro. Therefore, the use of animals will be restricted to address scientific questions that cannot be addressed using in vitro and ex-vivo settings.

A retrospective assessment of replacement will be due by 10 November 2028

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?

Calculations based on data obtained from published studies and/or in-house previous studies will be used to determine animal numbers. The approximation of the sample size for in vivo experiments was done by the G*Power 3.1.3 software.

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

Multiple approaches will be used to reduce the number of mice being used:

- Investigating the changes that occur in platelets and their interactions with immune cells during sepsis by human blood from healthy donors and septic patients and identification of possible target that thrombosis and inflammation under septic conditions.

- Potential drugs will be tested first in vitro with human blood to assess the doses required and its efficacy in reducing selective events.

- Once selected pathways are identified, the number of mice required will be calculated and powered.

- Mice will be randomised and blindly assigned to different groups where possible to minimise unintentional bias, unless when GA mice 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?

We will use well-designed breeding strategies to ensure the required genotypes are produced as efficiently as possible.

Pilot studies will be performed initially to inform future study design. Most tissues will be collected and stored for different analysis and organs will be made available for other labs/collaborators.

A retrospective assessment of reduction will be due by 10 November 2028

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 project, we will use 2 mouse models of sepsis. Our first model of choice will be intraperitoneal injection of LPS. We chose the intraperitoneal route over intravenous injection to reduce the severity of the inflammatory response. IP can still achieve the scientific outcome needed for our experiments. We will choose the lowest dose of LPS able to induce thrombosis in different organs within 48h.

In the second model (CLP), we aim to assess whether targeting the pathway identified using LPS model alters bacterial trapping in the clots and leads bacterial growth and dissemination . To refine CLP, we will perform a short ligation (approximately 1cm) and a single puncture to lower bacterial burden and the intensity of the systemic inflammatory

response, which is the main cause of pain in septic mice. This model will be used only following identification of key pathways using LPS model.

In both models, pilot studies will be performed to assess the shorter time needed to develop microvascular thrombosis, the main complication and our scientific target in this licence. These pilot studies will inform about the duration of sepsis that is required to reach the scientific outcome, within the permitted severity.

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

Sepsis leads to multiple organ damage. This is due to systemic dysregulated inflammation and microvascular thrombosis in multiple organs. This inflammatory reaction is triggered by the endotoxin LPS and its binding to cells through its receptor TLR4. Whereas some studies are possible in zebrafish and other less sentient species, we are not able to use these species for these particular studies as they lack the receptor TLR4 involved in the recognition of the endotoxin, suggesting that these models use alternative pathways relative to human to activate platelets and immune cells during sepsis. This limitation and inability to translate to human sepsis doesn't allow to perform the proposed models.

Therefore, mice are the first choice to perform this research.

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

First, as recommended by recent guidelines of RSPCA (Ref below), we will use LPS as a proof-of- concept to induce sepsis. We will assess the development of thrombosis is different organs following LPS. The CLP model will only be used in a staged manner where the LPS approach has generated results worthy of further investigation. Using LPS injection, mice will not undergo surgery as in the case of CLP. LPS induces a quick and acute inflammatory response, which will result in the development of microthrombi in multiple organs. We will first assess whether lower dose of LPS (lower than 10mg/kg) is able to induce thrombosis in multiple organs within 48h. The lowest dose to achieve multi-organ thrombosis will be used. CLP is closer to human sepsis which is progressive inflammatory response including the pathogen. As the aim of the project is to understand the contribution of the inflammatory response, independent of the pathogen, to thrombosis, CLP will be used to assess whether inhibiting thrombosis alter bacterial growth. Therefore, both models are required to achieve the scientific aims of this project.

In order to reduce pain and support the animals whilst they experience the adverse effects associated with the inflammatory during sepsis, mice will be given analgesia, supplement warmth and fluids during the procedures.

We will follow the recent guidelines of RSPCA on the 3Rs of animal models of sepsis.

- Analgesia and fluids will be used a default position
- Pilot studies will be performed first (3 mice per group)

- LPS is our first choice of sepsis able to assess the role of the acute inflammatory response in thrombosis.

- We will use platelet count and kidney damage (assessed by blood urea nitrogen) (assessed using blood sample) as endpoint which we have previously shown to be a reliable biomarker for sepsis.

- Following induction of Sepsis, we will be guided by the mouse clinical assessment scoring system (M-CASS) recommended by RSPCA (reference below, table 4)

REFINEMENT OF ANIMAL MODELS OF SEPSIS AND SEPTIC SHOCK, Lilley et al, Shock, 2015

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).

We will follow the guidelines from RSPCA on the three Rs in the use of animal models or procedures involving sepsis and septic shock. We have taken into consideration the recommendations of the RSPCA expert working group regarding sepsis research.

- https://www.rspca.org.uk/webContent/staticImages/Downloads/FOSSproject.pdf

- REFINEMENT OF ANIMAL MODELS OF SEPSIS AND SEPTIC SHOCK, Lilley et al, Shock, 2015

The resulting data will be published in Open Access Journals whenever the data is completed and in accordance with the ARRIVE 2.0 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 signing into the NC3R newsletter, attending seminars and conferences, as well as discussing with the NVS and NACWOs.

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 used to replace animal use. We are currently assessing the potential use of organoids to replace the use of mice.

If we undertake a systemic review, we will use SyR F the free online platform for researchers. https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf

We will also stay up to date with guidance published by RSPCA, the International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardisation Committee on the most refined experimental methods for haemostasis and thrombosis research. We have for example included fluid administration (twice a day) in septic mice and we observed a significant reduction in pain in mice during the protocol.

A retrospective assessment of refinement will be due by 10 November 2028

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?

15. Investigating the biology of malignant haematopoietic cells

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Blood development, Blood cancer, Epigenetics (heritable changes beyond DNA sequence), Blood Stem cells, Therapeutic agents

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.

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 purpose of this project is to:

use mouse models to understand the disease biology of a type of leukeamia (known as chronic myelomonocytic leukaemia, CMML)
identify and validate genes or pathways that CMML cancer cells rely on and,
test the efficacy of novel treatment options in CMML models.

A retrospective assessment of these aims will be due by 20 December 2028

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?

Chronic myelomonocytic leukaemia (CMML) is a rare blood cell disease characterized by nonfunctional monocytes (a type of white blood cells that protect the body from foreign substances). Blood stem cell (cells that can generate all types of blood cells) transplantation is the only available option for CMML treatment. However, old age and lack of suitable donors are major obstacles to this treatment. Nearly all CMML cases are difficult to treat using standard therapies, and this is mainly due to the poor understanding of the basic mechanisms that drive disease development. The objective of our research is to understand how disease starts and progresses using mouse models, which may reveal novel ways to treat CMML patients. Potential treatments will be tested in leukaemia mouse models prior to testing them in clinical trials. Understanding CMML disease progression will also reveal novel strategies to prevent transition from a precancerous stage to chronic leukaemia stage (CMML) and from a chronic stage to acute leukaemia (AML).

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

Expected outputs include new knowledge about which genes and cellular pathways are most important in leukaemia and discovery of new treatment targets to develop into the clinic for patient benefit in the longer term.

The output of the research will be shared with other researchers in the field and the broader community through presentations in conferences (nationally and internationally), preprints and publications in peer-reviewed journals. We will also aim to present our work to lay audience at science outreach events. In addition, our work will produce applicable results that benefit leukaemia patients and we will investigate through collaborations with clinicians at the Establishment and other medical cancer centres worldwide.

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

The expected benefits of this project include new knowledge regarding the requirement for genes or cellular pathways for the function of blood cancer stem cells and monocytes; identification of new drug targets for the treatment of blood cancer; and evaluation of the efficacy of novel candidate drugs in experiments using mice. In the long term, novel treatment options are useful to treat CMML patients whose prognosis is dismal due to lack of effective treatment options. New knowledge gained from this work will also be applicable to other types of leukaemia which share similar features.

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

Our findings will be made available to other scientists through collaborations, publications in high profile peer-reviewed journals and presentations at scientific conferences and meetings. Our establishment has a policy of ensuring that all publications generated are available with free access to all.

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.

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 regarding their blood forming system. Only a mammalian blood cell generation model system has the potential to accurately mimic both the anatomy and complex cell biology, including microenvironmental interactions, of human normal and leukaemic blood cell generation.

Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human diseases of the blood and many reagents exist for the observable characterisation of mouse cells.

The human and mouse DNA content and organization are approximately similar, and display an equivalent number of genes, which share similar functions. Further, mice have genes not represented in other animal model organisms (e.g. nematode worm, and fruit flies) such as those involved in preventing similar infections in future following initial exposure. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field. Definitively, mouse models are important for placing the findings of in vitro (test tube) studies or comparative analysis of human samples into an appropriate and meaningful in vivo (living organism) context. It is the combination of in vitro, in silico (computer) and in vivo studies that provides the insight needed to understand cancer biology and develop new therapeutic approaches, and there are no effective approaches to hand that can replace the in vivo studies, as these allow the in vitro findings to be tested in an appropriate living organism. For our studies we need animals with a functionally mature immune system, therefore we will only use adult mice.

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

Mice will be transported to the research facility and housed in individually ventilated cages containing environmental enrichment. Mice will be treated once with X-rays (2-9 Gy) (60-75% of the mice) prior to transplantation to prepare them for receiving human or mouse normal or cancer cells injected into the blood or bone marrow. Mice may also be given drugs (both standard and novel) and at set periods of time blood samples will be taken. The blood samples will be used to study leukaemia onset and progression. Additionally, certain genes might be switched on or off by administration of hormones/small molecules to the mice using various routes depending on the compound administered. Occasionally, mice will be injected with a label following inhalation anaesthesia to monitor tumour cell distribution across the body. At the end of the study the mice will be killed humanely, and blood / organs removed for study. Most procedures in the protocols, such as X-ray treatment of mice, blood or bone marrow sampling, or injection of cells and drugs are not associated with suffering that lasts for longer than 24 hours. Mice injected with leukaemia

cells will, when the disease develops, exhibit signs of disease, such as hunched posture, bristling of hairs and poor levels of socialising and interaction. Under these circumstances, and whenever else an animal displays features of ill health, or at the end of each experiment, mice will be humanely culled.

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

Many of the mice will show signs of leukaemia such as loss of body weight, enlarged spleen, hunched posture and bristling of hairs. Mice will be inspected daily for loss of body condition and / or signs of significant suffering. Some mice (<5%) may develop fatal aggressive leukaemia within just 1-2 days. These mice with aggressive leukaemia may be found to die overnight when they appear to be in full health the day before. This is typical of patients with leukaemia.

Experimental procedure mice undergo for receiving leukemic cells using X-ray, can result in potential side effects such as reduced mobility, dehydration, and lack of appetite. Consequently, mice may be expected to show transient weight loss.
Mice receiving hormones to induce deletion of genes may experience reduced mobility and transient weight loss.

Mice injected with blood cells into the thigh bone may have trouble in knee movements and exhibit a limp for up to six hours and then recover with no adverse effects.
Mice will experience transient discomfort during 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)?

Based on our experience using these procedures and experimental models we anticipate about 85% of mice to experience Moderate severity (no worse than regular injection / administration by mouth; loss of up to 20% of bodyweight), 10% Mild ((no worse that the occasional injection of agents / cells), and less than 5% a Severe one (found dead overnight when observed to be healthy the day before). Some mice will experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery. We will aim to utilise the least stressful route of administration wherever possible.

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

- Killed

A retrospective assessment of these predicted harms will be due by 20 December 2028

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?

CMML is a stem cell disease. Stem cells are defined by their ability to regenerate normal or leukaemic bone marrow cells following their transfer in to a second animal. Cell culture experimental systems are insufficient for this purpose because they do not provide the required cellular and recipient interaction for the development of a new tumour or normal bone marrow system. Cell culture cannot currently replicate the complex cell structure involving interactions between many different cell types. Thus, without the use of a live, whole animal experimental system, the biology of bone marrow stem cells cannot be studied meaningfully.

Similarly, therapeutic targets that show efficacy in terms of cell viability in cell culture system many times do not show any efficacy in animal models due to lack of microenvironmental cues. Therefore, use of experimental models of leukaemia is essential for testing therapeutic targets.

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

We have considered using patient samples for this project. Due to rarity of the disease, sample availability is a limiting issue. However, drugs will be tested on selected patient samples in vitro prior to testing them in vivo where possible.

Samples from genetically engineered mouse models or mouse models that received patient cells (patient xenografts) will be used evaluate the efficacy of drugs prior to testing them in vivo.

Other model organisms such as fruit flies or fish do not accurately recapitulate the mammalian blood development system.

Why were they not suitable?

Currently there are no cell culture systems available to study CMML. In addition, one of the drawbacks of experiments using human leukaemia cell line is their genetic variability making the accurate and meaningful study of the effects of specific genetic lesions in isolation very difficult. By contrast, mouse models of human leukaemia enable investigation of the biological effects of specific genetic lesions in a tractable, controlled and highly informative manner.

A retrospective assessment of replacement will be due by 20 December 2028

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 leukaemia or leukaemia incidence following manipulation of a gene of interest or treatment with a drug can be determined using the minimal number of animals.

Based on experience, group sizes of 5-10 animals (dependent on the readout) per experimental group suffice. A statistician has helped us to calculate the minimum numbers required. For instance, in implantation experiments where we deplete a gene in a mouse stem cell by genetic manipulation in vitro, we will use two independent approaches targeting the gene as well as a control for that approach. Moreover, we would typically examine more than one model cell subpopulation. Likewise, we may use several doses of a drug, or several different drugs or treatment combinations to test a hypothesis. We have estimated the total number of mice to be used over the licence lifetime considering our previous experience of my project licence.

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

Use of mice will be minimised by (i) making use of relevant cell culture systems wherever scientifically justified, (ii) use of bioimaging live animals or bone marrow sampling to follow disease development and response in real time (rather than killing groups of mice at defined time points), (iii) careful experimental design informed by the expert advice of a statistician (consulted regularly) so that the minimum number of mice required to produce a scientifically acceptable result are used, (iv) the use of small 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 leukaemia samples (which eliminates a requirement for continuous production of groups of mice with experimentally initiated CMML).

The use of mice will be minimised in several other ways:

By considering on-going statistical estimation of power requirements in each of the studies, using prior results to use the minimum number of animals while retaining sufficient numbers for statistical significance. In general, we will use a sample size capable of detecting a 30% practical difference with 80% power and 95% confidence.

By incorporating as many test groups as possible within a single controlled experiment, reducing the number of controls required compared to a series of smaller experiments.

By utilising tissues and tumours from different sites on one mouse for both treatment and control samples.

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 optimize the mice in several ways:

By optimising our breeding programme to maximise use of mice in experimental protocols minimising the number of mice we do not require. Breeding is performed in a different PPL, by a dedicated group in our Establishment.

By doing as much preliminary work as possible in culture models in vitro and in silico analysis prior to engaging in in vivo studies.

By minimising variability in results through utilising inbred strains and by housing them under identical conditions to limit variability.

By performing pilot studies using few mice when no information is available in the literature so that the number of mice utilised in experiments is reduced to minimal levels.

In all new experimental models and protocols, we will establish the baseline by procuring help and advice from Biological Research Unit staff and researchers at the Establishment but also from our experienced collaborators outside across the UK and internationally.

Furthermore, we will design small pilot experiments, carried out referring to https://www.nc3rs.org.uk/conducting-pilot-study, that will allow us to select the ideal cell line so fewer animals are used, to calculate the minimum cohort size given the rate of expected events and to determine gravity of these, allowing more accuracy for statistical powering calculation of group sizes in potential repeats.

A retrospective assessment of reduction will be due by 20 December 2028

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 about their blood forming system.
The techniques used have been carefully evaluated to minimise distress to the mice. X-ray doses will be administered at a level sufficient to induce bone marrow suppression but no other long-term adverse effect; bone marrow injections and aspirates will be not performed routinely, only where the scientific justification is high; and in studies that result in the initiation of leukaemia, mice will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed. We will use Establishment best practices for housing, dosing, and blood sampling of mice.

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

Only a mammalian blood generating model system has the potential to accurately mimic both the anatomy and complex cell biology, interactions, of human normal and leukaemic blood cell generation.

Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human blood cell diseases and many reagents exist for the characterization of mouse cells.

Less sentient animals do not exhibit a similar microenvironmental cues such as presence of niche cells or tissue rigidity and histopathological features as ageing humans. Mice are far more similar to humans than other animals and this is critical both for using reagents like drugs developed for human targets 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 possible.

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

The techniques used have been carefully evaluated to minimise distress to the animals, such as use of either tunnel handling or cupping for handling mice. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term impact; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; and in studies that result in the initiation of leukaemia, mice will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed.

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

Surgical procedures will be carried according to the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2017.

Unless otherwise specified, this project will follow the "Guidelines for the welfare and use of animals in cancer research" and the administration of substances and withdrawal of blood 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 (Morton et al., Lab Animals, 35(1): 1-41 (2001); Workman P, et al. British Journal of Cancer, 102:1555-77 (2010)).

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 animal care and welfare officer, and vet. I read the quarterly reports produced by our Named Information Officer (NIO). I am a regular attendee and contributor to our Retrospective Review and Licensees meetings and the 3Rs Poster session, all of which take place annually at our Establishment.

A retrospective assessment of refinement will be due by 20 December 2028

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?

16. Biology of normal and leukaemic cells

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

therapy, haematopoiesis, cancer, leukaemia, stem cells

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.

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 research aim of the project is to unravel the underlying mechanisms during normal blood formation and blood cancers development, in order to establish a sound, biological rationale for explaining underlying transformation mechanisms and hence for developing novel therapeutic intervention.

A retrospective assessment of these aims will be due by 21 December 2028

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?

Blood cancer affects human from infant to elderly, and the overall survival of the patients are poor, in particular to patients with acute leukaemia. Better therapeutic intervention is warranted to improve the welfare of the patients. Pre-clinical study using animal model provides invaluable scientific data to justify and develop novel therapeutic intervention.

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

New knowledge on how the gene regulating normal blood formation and the blood cancer proliferation and development, biologically and biochemically.

New knowledge on what and how anti-cancer substances eliminate blood cancer, biologically and biochemically.

The new knowledge generated will be published in peer-review scientific journals, such the knowledge can be shared with public and peers in the field.

Patent may be produced from the discovery of novel treatment of blood cancer.

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

In short-term, the scientists will be benefited from the new knowledge generated and advanced the understanding of blood formation and development of blood cancer. If the students work in the project, the science knowledge and research skill will be developed. In longer-term, until the project completed or beyond, new approach to eliminate particular types of blood cancer could be developed, based on the knowledge generated in this project. This provides the opportunity of clinical trial and potentially better cure and care to the patient with blood cancers.

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

Any output of this work will be published to peer-reviewed scientific journals with open access, therefore anyone interested to the topic can be benefited.

This project is not only within the UK, but in the collaboration with the scientists in the other regions, for example Germany, Switzerland, the US and Hong Kong.

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.

Mice are the lowest mammals in which the haematopoietic development systems have been well characterized. They are also widely used and accepted models for this application. Moreover, overwhelming amount of evidence and previous experimental data demonstrate striking similarity between mice and humans in terms of haematopoiesis. The haematopoietic system in mice can easily be fully reconstituted, and manipulated to allow human haematopoiesis. With optimized conditioning protocols, most of the animals will survive, and live to their full life span without ever showing signs of illness. Thus we believe that the mouse model is the least severe one that would produce satisfactory results.

The experiments could be performed when the mice reach 6 weeks old or beyond. This is the best age for experiments because their blood formation system are fully developed, and the size of the animals at this age allow researcher easier to perform any injections and surgical procedures, therefore the harm/ discomfort to the animals can be minimized.

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

Depends on the purpose of the experiments, the duration of the experiments could be from a few days up to maximum 2 years. The animals will usually receive irradiation to allow transplantation, injection of cells via blood vein or tummy or into the bone marrow. Administration of gene alternation and/ or anti-cancer substances orally, or via injection of blood vein and tummy. Finally, the experiment mice will be culled in humane way. Irradiation and injection of cells will only be done once to the animals. Typically administration of gene alternation substances are from 3 days to 2 weeks; administration of anti-cancer substances are from 3 days to 1 months. If the animals receive injection under skin, into the bone, or for bioimaging, they will be put under light anaesthesia to minimize the pain and their movement. For identification purposes, the small piece(s) of ear skin will be notched. Depends on the experimental design, typically one animal will receive one irradiation, one injection of cells, 3 to maximum 10 injections for gene alternation substances, 3 to maximum 20 injections for anti-cancer substances. If the experiments are not for anti-cancer purposes, typically the animals will receive average 5 but less than 10 injections; If for anti-cancer purposes, they will receive 15 injections in average. All animals will be killed by the end of the experiments.

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

Animals normally would not suffer from any distress if they are used for breeding maintenance; Animals with harmful phenotypes may be born smaller and weaker than their normal littermates.

Some animals (in about 1 in 100), will develop skin and hair conditions e.g. barbering, patch baldness, and eczema, which may lead to excess scratching and bleeding which may be treated with wound powder for up to 1 week, if the treatment does not improve symptoms or if more than 5% of the skin is affected the mouse should be culled.

Therefore, the animal should experience this kinds of distress no more than 1 week. If skin irritation is caused by fighting with secondary bacterial infections, mice may be treated with topic and oral antibiotic (after consultation with the VET), and the aggressive mouse will be removed immediately.

For the animals used for experiments, following irradiation, animals may show some loss of appetite, weight loss and loss of condition. At the irradiation dose optimized and

specified, less than 5 in 100 mice are expected to be adversely affected. These should last no more than 1 week from irradiation and the mice will recover.

If the mice are injected with substances via tail vein or abdomen, or withdrawal of blood via tail vein, mice may experience short term discomfort after injection, and recover to normal within 1-2 days. Pain may be controlled if evident by pain killer (analgesic) (such as NSAIDS). No adverse health effects are anticipated in the indicated period following injection.

If the mice are injected with substances via bone marrow cavity, or under their skin, or subjected to imaging, they will experience short-term anaesthesia no more than 15 minutes to restrain their movement for injection/ imaging. The mice may experience dizzy for 5-10 minutes after withdrawal of anaesthesia , and fully recover without distress associated to anaesthesia.

In the recovery period from bone marrow injection, animals will experience abnormal limb movement, pronounced lameness for about 3 days. Pain killer will be administered following advice from the Vet. Effectiveness of pain killing will be judged by if normal behaviour returns.

After the transplantation, during the development of cancer any animal will gradually experience weight loss, hind limb mobility issues, such that animals have difficulty moving around cage and reaching food and water, lameness or non-weight bearing limbs, or palpable tumours, pathological bone pain will manifest as loss of mobility whereby the animal cannot walk freely enough to reach food or drink, and/or other common signs of distress (less active, hunched posture, loss of physical/mental alertness, and/or loss of self-grooming). Mice will be monitored for these symptoms daily and culled if these signs are observed. The animals may experience this level of distress 1-2 days, the researchers will cull the animals once symptom(s) mentioned found.

On rare occasions (approx. 2 in 100) sudden paralysis may occur, i.e. over-night and not a result of preceding movement difficulty and these animals will be immediately culled. Therefore animals would not suffer from this condition for more than 1 day.

Sudden death may occur due to leukaemia.

Diarrhoea is not expected to occur in animals that are immune competent, and on the rare occasion that it does if symptoms do not improve within 24 hours animals will be culled.

Therefore animals would not suffer from this condition for more than 1 day.

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 sub-threshold (43%); mild (30%); moderate (20%); severe (7%).

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

- Killed

A retrospective assessment of these predicted harms will be due by 21 December 2028

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?

There are important technical aspects of manipulating gene expression to identify key steps in blood cell production or blood cancer development where in vivo work is necessary to model the disease as whole animal, as these processes are very complex, involve multiple tissues and organs (e.g. bone marrow, spleen, lymph node, thymus etc.) and interact each other in situ and in real time manner. These complex interactions cannot be reproduced in test tube and outside the animals. And to build a coherent picture of the molecular genetics and natural history of leukaemia using patient samples but the veracity and credibility of the models we construct would benefit greatly from the stringency of animal modelling which we can do in mice.

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

We use human cancer cell lines and patient samples and culture them in vitro. The key gene of interest are targeted by chemical and genetic approaches and the effects of manipulation are assessed biologically and biochemically. These experiments are only reliable in short-term (days/ weeks). Organ-on-a-chips were also considered, however this technique is still not mature to mimic complex organs/ tissues interactions in animals during normal blood formation and blood cancer development.

Why were they not suitable?

long-term monitoring (months) of the disease development using patient samples in test tube is still technically impossible, a lot of artifacts produced if culturing in long-term and significantly skewed the data.

physiological normal haematopoiesis and leukaemogenesis requires multiple organs and very complex niches to maintain the stem cells and direct their differentiation. It is still technically impossible to mimic such complex niches ex vivo.

Potential adverse effects of chemical and/or genetic manipulation as whole organisms cannot be observed in vitro/ ex vivo.

A retrospective assessment of replacement will be due by 21 December 2028

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?

Number of animals to be used consists of breeding and maintenance of genetic altered animals (GAA) (protocol 1 and 2), and for experiments (protocol 3-6).

For the breeding and maintenance of GAA, the number of animals used is based on the optimized breeding strategy to minimize the culling of unwanted genotyping animals; This project will involve about 50 GAA colonies and about 400 mice each colony will be used for breeding and maintenance during 5 years. Total 20,000 mice.

Animals are required to avoid genetic drift causing by inbred of GAA colonies (usually after 10 generations) by back-cross with wild-type animals and subsequent matings to retrieve desirable genotype. Therefore, each colony requires about 1-2 backcross within 5 years; Most of the GAA colonies in this project contains 2-4 transgenic alleles, therefore at least 2-3 generations are required to retrieve desirable genotype; About 5000 mice are required for this purpose.

For the use of animals in experimental protocols, the principles of the experimental design to fulfil the project aim and objectives are - (1) Comparison of the effect to normal and malignant haematopoiesis without (control group) or with genetic alternation (experimental group); (2) Comparison of the effect to normal and malignant haematopoiesis without (control group) or with anti-cancer reagents treatment (experimental group); (3) Comparison of the effectiveness of in vivo reconstitution of normal haematopoiesis or leukaemia arise from different cell counterparts (for example: sub-population of haematopoietic stem or progenitor cells from bone marrow)
The control group is used as the reference point to evaluate the effect of genetic alternation or treatment intervention. To minimize the use of control group, whenever possible, we will pool multiple single variable experiments into one, therefore multiple experiments can share the data from one control group. For example, if 3 drugs need to be tested, when possible, they will arrange into 4 groups (1 + 3). Animals from both sexes will be used to eliminate the sex bias and also reduce waste of animals from one sex. The group sizes are determined by online POWER tool. Depends on the variation of the groups, and from experience of past experiments, typically a sample size of 10-20 animals are required to produce statistics significant data. Whenever the scientific judgement/ ethnicity can uphold, we will use the data from previous work from our group or from published literature, to avoid the repeat of the experiments. We always first do the literature search to estimate the variation of the experiments and determine the most appropriate dose when anti-cancer reagents to be used. If the information from previous work or published literature is not sufficient to make a sound decision to decide a group size, we use a small cohort of animals (usually 3 animals a group) as pilot study to estimate the difference of two means and variation (effect size), and use the data from

pilot study to decide the sample size. The groups are usually compared by unpaired or paired T-test, log-ranked test, ANOVA test etc, with the assistance of programme like EXCEL or PRISM.

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

All assays are carried out in vitro and results assessed before continuing onto in vivo analysis.

Statistic analysis (e.g. POWER) to estimate minimum number of animals required in each group to achieve the comparison with statistically significant.

We will use the same control groups for multiple experiments wherever possible, e.g. same control for heterozygous and homozygous mice or for mice treated with different anti-cancer drugs.

Whenever possible, we label the cells with fluorescence/ luciferase and the disease development in vital organs can be monitored by bio-imaging system at multiple time points, therefore reduce the animal numbers used from killing each 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?

Wherever the welfare of the animals and scientific data are not compromised, genetic altered animals are breeding in homozygous, therefore unwanted genotype animals would be minimized.

Moreover, to further minimise the number of mice used in this project, we use wherever possible littermates carrying no genetic alteration that are generated in our breeding programme as they represent the ideal controls for genetically modified mice.

When tissues required for experiments, whenever possible, the tissues would be shared for different experiments by different researchers within/ outside the group.

Whenever possible without compromising the quality of science, data from the old in vivo experiment could be combined and/ or re-analysed to reduce the number of animals used in new experiment.

A retrospective assessment of reduction will be due by 21 December 2028

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 lowest vertebrate group in which the haematopoietic development systems have been well characterized. They are also widely used and accepted models for this application. Moreover, overwhelming amount of evidence and previous experimental data demonstrate striking similarity between mice and humans in terms of haematopoiesis. The haematopoietic system in mice can easily be fully reconstituted, and manipulated to allow human haematopoiesis. Therefore, transplantation via tail vein or directly into the bone marrow is the “golden standard” to evaluate and study the development of normal haematopoiesis and leukemogenesis in vivo for more than two decades.

The transplantation technique we are using is the most refined and widely used and published among peers internationally. Moreover, the technique to modify mouse genome (genetic alternation) has been developed more than three decades and the technique is now very matured, reliable and robust to induce gene alteration, including gene activation, deletion, point mutation, knock-out and knock-in, with resources and technical support available nationally and internationally. With optimized conditioning protocols, most of the animals will survive, and live to their full life span without ever showing signs of illness. Thus we believe that the mouse model is the least severe one that would produce satisfactory results and fulfill the aim and objectives.

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

Haematopoiesis system of immature life stage (embryo; primitive haematopoiesis) is different from postnal and adult stage (definitive haematopoiesis). E.g. the haematopoietic stem cells of embryo are in yolk sac and later foetal liver, but in bone marrow when developed to postnal and adult stage. Transplantation experiments cannot be performed in embryonic stage but in adult stage.

Zebrafish is a vertebrate considered less sentient than mice, and an alternative model used in haematopoietic study in science. However, the haematopoietic system of zebrafish is less conserved with human compared with mouse. And unlike mouse, zebrafish cannot be served as recipient of human cell lines/ patient samples, therefore zebrafish cannot be used as an humanized animal model.

Normal haematopoiesis/ leukaemia development require at least weeks to months, therefore it is impossible to use terminally anaesthetised animals for experiment.

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

All animals are monitored daily by the researchers and the staff of biological service to ensure the welfare of the animals is uphold and would not exceed the severity limit. Any animal has health concern will be communicated between researchers and BSU staff therefore these animals will be attended particularly (e.g. check twice a day, daily body weight measurement). Whenever surgery involved to the animals, pain killer would be used before and after the procedure, until they are fully recovered, and shown no distress even pain killer withdrawn. If they have temporary difficulty to access food and water (e.g.

after bone marrow injection), wet food (nutri-gel) will be provided. The least invasive approach possible will be employed in all experiments with scientific justification (e.g. intravenous injection for transplantation is the first attempt, instead of more invasive intrafumoral injection; blood sampling and bioimaging instead of killing the animal for tissues). All animals for breeding and maintenance of the colonies will not reach their old age (over 12 months old). For experimental purposes, normally the experimental mice will not reach their old age; A small number of animals may need to keep the mice over 12 months old, due to the study of normal blood formation of their whole natural life span, or blood cancer development is long. In this case, they will all be closely monitored if they suffer from any age-related clinical signs, and will be responded according to the protocols.

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

We refer to (1) Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 from the Home Office; (2) ARRIVE Guidance 2.0 from NC3Rs; (3) UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia (Second Edition) from British Journal of Cancer; (4) Guidelines for the Welfare and Use of Animals in Cancer Research from British Journal of Cancer; (5) Published resources from https://www.nc3rs.org.uk/

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

We have regular meetings with the Biological Service Unit will provide update in 3Rs, and have a NC3Rs Regional Programme Manager. We attend the workshop organised by the NC3Rs manager, that provides update and advise on 3Rs.

A retrospective assessment of refinement will be due by 21 December 2028

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?

17. The biology of normal and malignant haematopoietic cells

Project duration

5 years 0 months

Project purpose

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

Key words

Leukaemia, stem cells, haematopoiesis

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.

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?

To evaluate the role of genes and cellular pathways that drive growth of cells in a type of blood cancer called acute leukaemia but which do not affect the growth of normal blood cells, so as to generate better therapies for patients with blood cancer.

A retrospective assessment of these aims will be due by 21 December 2028

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?

There is a need for new treatments for patients with blood cancer as well as a need for greater understanding of disease processes which enable the design of new treatments. In particular, acute leukaemias are driven by disease-causing cells which must be completely eliminated in order to cure patients. The purpose of this project is to develop greater understanding of these leukaemia diseasecausing cells and to determine how they differ from normal blood forming cells. Through understanding these differences we aim to develop new knowledge that will facilitate the development of treatments for the benefit of patients which will take place under the auspices of this licence, as well as by researchers elsewhere in the future.

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

Expected benefits include new knowledge about which genes and cellular pathways are important in leukaemia and discovery of new treatment targets that may deliver patient benefit in the longer term.

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

The expected benefits of this project would include:

New knowledge regarding the requirement for several genes and cellular pathways for the function of malignant blood cancer cells, with a particular focus on those selectively required for the maintenance of leukaemia-causing cells but not normal blood cells. Identification of specific cellular targets in blood cancers which would be of particular interest to academic researchers, the pharmaceutical industry (with regard to drug development) and clinicians treating patients.

Evaluation of the effect of one or more candidate therapies on normal and leukaemic blood forming tissue which would again be of particular interest to the pharmaceutical industry (with regard to drug development) and clinicians treating patients.

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

The output of the research will be shared with other researchers in the field and the broader community through presentations in conferences (nationally and internationally), publications in peerreviewed journals, and with the public through appropriate social media channels.

In addition, our work has direct translational and clinical applications that we will investigate through collaborations with researchers and clinicians at the Establishment and other medical cancer centres worldwide.

Our Institution has a policy of ensuring that all publications generated are available for all to access freely.

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.

Mice have been chosen for this research because as mammals they have genetic and biological similarities to humans with regard to their blood forming system. Only a mammalian blood cell generation model system has the potential to accurately mimic human normal and leukaemic blood cell production. Furthermore, there is extensive experience in the wider scientific community regarding the use of mice as a model system for human diseases of the blood.

The mouse is one of the model organisms that most closely resembles humans. Humans and mice display an equivalent number of genes, which are similar in function. Further, mice have genes not represented in other animal model organisms (e.g. fruit fly, worm) such as those involved in immune responses. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field.

Mouse models are important for placing the findings of test tube studies of human samples into an appropriate and meaningful living organism context. It is the combination of test tube, computer and living organism studies that provides the insight needed to understand cancer biology and develop new therapeutic approaches, and there are no effective approaches that can replace the studies in mice. This is because these studies allow the test tube findings to be evaluated in an appropriate environment within a living body. For our studies we need animals with a functional immune system, therefore we will only use adult mice.

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

A typical experiment, representative of around three quarters of experiments performed on our previous licence over the last five years would involve X-ray treatment of mice followed by injection of blood cancer cells into the tail vein, administration of antibiotics in the drinking water and blood count monitoring every four weeks until the mice are humanely killed four months later. Some experiments would, in addition, involve treatment of mice with drugs by mouth typically for daily for 2-3 weeks.

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

The proposed procedures, such as X-ray treatment, anaesthesia, injections and blood sampling are mild in severity and are associated with at worst, only slight or transitory and minor pain or suffering. Occasionally (5-10% of procedures) after full dose irradiation some mice might lose weight for several days before regaining it and so these procedures are considered moderate in their severity. Additionally, infrequently some mice will experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery. We always aim to utilise the least stressful route of administration wherever possible. Mice

injected with blood cancer cells will, when the disease develops, exhibit signs of disease, such as hunched posture, poor levels of socialising and interaction. Under these circumstances, and whenever else a mouse displays features of ill health, or at the end of each experiment, mice will be humanely killed using a Home Office sanctioned 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)?

Based on our experience using these procedures and experimental models we anticipate about 8590% of mice experience mild severity, 5-10% moderate and 2-5% a severe one.

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

- Killed

A retrospective assessment of these predicted harms will be due by 21 December 2028

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?

As described above, mice have been chosen for this research because as mammals they have genetic and biological similarities to humans with regard to their blood forming system. Only a mammalian blood cell generation model system has the potential to accurately mimic human normal and leukaemic blood cell production. The mouse is one of the model organisms that most closely resembles humans. Humans and mice display an equivalent number of genes, which are similar in function. Further, mice have genes not represented in other animal model organisms (e.g. fruit fly, worm) such as those involved in immune responses. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field.

Mouse models are important for placing the findings of test tube studies of human samples into an appropriate and meaningful living organism context. It is the combination of test tube, computer and living organism studies that provides the insight needed to understand cancer biology and develop new therapeutic approaches, and there are no effective approaches that can replace the studies in mice. This is because these studies allow the test tube findings to be evaluated in an appropriate environment within a living body.

Test tube experimental systems, and computer modelling systems, are insufficient for this purpose, because they do not provide the required cellular and host environment for the

development of a novel blood cancer, or normal blood system, which typically has a complicated architecture, involving interactions between many different cell types.

Representative of this is the finding that some cells which seem to have long lasting activity in test tube experiments in the laboratory do not have the same properties when tested in a living system; it is the cells that are long lived in a living system which this project aims to investigate and understand. Thus without the use of a live, whole animal experimental system, the biology of normal and blood cancer initiating cells cannot be meaningfully studied.

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

Laboratory test tube experiments and computer modelling systems.

Why were they not suitable?

As described above, only a mammalian blood cell generation model system has the potential to accurately mimic human normal and leukaemic blood cell production.

A retrospective assessment of replacement will be due by 21 December 2028

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 blood cancer cell burden or incidence following manipulation of a gene of interest or treatment with a drug can be determined using the minimal number of animals.
Based on past experience, group sizes of between 5 and 10 animals (depending on the investigation) per experimental group suffice. For instance, in experiments where we deplete a gene in a cell type by genetic manipulation in the test tube, we might use two independent approaches targeting the gene as well as a control for that approach, and potentially several doses of a drug, or several different drugs or treatment combinations to test a hypothesis. We have estimated the total number of mice to be used over the licence lifetime taking into account our previous experience of my 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 mice will be minimised in several ways:

By considering ongoing statistical estimation of power requirements in each of the studies, using prior results in order to use the minimum number of animals while retaining sufficient numbers for statistical significance. In general, we will use a sample size capable of detecting a 40% practical difference with 80% power and 95% confidence.

By incorporating as many test groups as possible within a single controlled experiment, reducing the number of controls required compared to a series of smaller experiments.

By utilising tissues and tumours from different sites on one mouse for both treatment and control samples.

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 mice will be optimised in several ways:

By optimising our breeding programme to maximise use of mice in experimental protocols thereby minimising the number of mice we do not require. Breeding is performed on a different project licence by a dedicated group in our Establishment.

By doing as much preliminary work as possible in culture models in vitro and in silico analysis prior to engaging in in vivo studies.

By minimising variability in results through utilising inbred strains and by housing them under similar conditions .

By performing pilot studies using few mice when no information is available in the literature so that the number of mice utilised in experiments is reduced to minimal levels.

In all new experimental models and protocols, we will establish the base line by procuring help and advice from animal care and veterinary staff and researchers at the Establishment but also from our experienced collaborators outside across the UK and internationally. Furthermore, we will design small pilot experiments, carried out referring to https://www.nc3rs.org.uk/conducting-pilot-study, that will allow us, where appropriate, to select the ideal cell type so fewer animals are used, to calculate the minimum cohort size given the rate of expected events and also to determine gravity of these events, allowing more accuracy for statistical powering calculation of group sizes in potential repeats.

Lastly, pilot experiments, potentially for all aspects of the Protocols, will enable us to better plan the length and size of the experiment and to help monitor for side effects at the critical time points.

A retrospective assessment of reduction will be due by 21 December 2028

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 transplantation and drug treatment models are evaluated repeatedly to optimise animal welfare. Establishment staff monitor mice regularly with weights and observations, and this occurs more frequently following any procedure (e.g. daily weights). In the small proportion of instances where mice have lost weight (e.g. after higher doses of X-ray irradiation) mice are offered mash in addition to their usual diet and monitored more frequently (e.g. twice daily). Mice occupy enriched environment and are cared for by staff trained and expert in animal monitoring and handling. For experimental approaches the least invasive route for drug treatment is always followed where possible (e.g. drinking water versus intravenous or intraperitoneal where a drug is orally bioavailable and stable); mice who undergo anaesthesia and bone marrow puncture are treated with fluid and analgesia to minimise risk of distress.

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

Other less sentient non-mammalian species, such as zebrafish or Xenopus, which lack a haematopoietic system that is comparable in complexity and anatomy to that of humans have been considered and rejected as models. Only a mammalian blood system has the potential to accurately mimic both the anatomy and complex cell biology, including microenvironmental interactions, of human normal and blood cancer cell tissue. One of the drawbacks of experiments using human blood cancer cells is their genetic heterogeneity making the accurate and meaningful study of the effects of specific genetic lesions in isolation very difficult. By contrast, murine models of human leukaemia enable investigation of the biological effects of specific genetic lesions in a tractable, controlled and highly informative manner. Adults need to be used because they have a fully developed immune system whereas mice at an earlier stage of development do not. There is no published experience to date on the xenotransplantation of human haematopoietic cells into fish or amphibians.

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

The techniques used have been carefully evaluated to minimise distress to the animals. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term impact; higher doses of X-ray irradiation are delivered in split doses; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; and in studies that result in the initiation of blood cancer, mice will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed.

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

The techniques used have been carefully evaluated to minimise distress to the animals. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term impact; higher doses of X-ray irradiation are delivered in split doses; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; and in studies that result in the initiation of blood cancer, mice will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed.

Where we use aged mice we will refer to Wilkinson (Laboratory Animals 2020, volume 5(34)), relating to the husbandry and care of aging mice.

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, and through discussing refinements with our animal care staff and vet. I will also read the NIO reports that are circulated within the Establishment. I am a regular attendee and contributor to our Retrospective Review and Licensees meetings and the 3Rs Poster session, all of which take place annually at our Establishment.

A retrospective assessment of refinement will be due by 21 December 2028

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?

18. Mechanistic studies in mouse models of cerebrovascular disease

Project duration

5 years 0 months

Project purpose

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

Key words

Cerebral artery disease, Stroke, Vascular dementia, Treatment

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

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?

To use mouse models to acquire a better understanding of what causes damage to the brain and its blood vessels during stroke or vascular dementia to allow the development of new treatments.

A retrospective assessment of these aims will be due by 23 December 2028

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 consequences of diseases of brain blood vessels are among the leading health issues globally. Stroke or "brain attack" is perhaps the best-known consequence, however, diseases of brain blood vessels are also important causes of dementias and resultant memory loss. Despite their importance, our understanding of what causes these diseases is poorly understood. Furthermore, there is a lack of effective treatments. The purpose of this project is therefore, to identify the biological compounds and cells involved in initiating, perpetuating, or aggravating the disease process. These studies should ultimately identify novel drugs for the treatment of common diseases affecting the brain.

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

A number of outputs will be gained from this work. It will firstly provide new and important scientific knowledge regarding the biological compounds and cells that cause brain blood vessel diseases. This knowledge will be disseminated to the scientific community through the publication of research articles and through presentations at scientific conferences. Ultimately, this knowledge will reveal new drug targets for the long-term development of drugs to prevent and/or treat brain blood vessel diseases in humans.

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

The benefits of this programme of work will be to the advancement of scientific knowledge (shortmedium term), to patients with cerebrovascular disease, e.g. stroke and vascular dementia (long-term), and to clinicians working in this area and treating these patients (long-term).

Short/medium-term benefits
Scientists and clinicians will benefit from these outputs as this work will provide a greater understanding of the biological compounds and cells responsible for some diseases affecting the brain. It will also enable researchers to identify new avenues for further investigation.

Long-term benefits
The outcomes of this work may have long-term benefits for patients and clinicians in terms of the identification and development of new drugs to prevent or treat stroke and vascular dementia. Furthermore, by identifying new biological compounds, this work has the potential to assist medical doctors in the identification and monitoring of patients who are at risk of having a stroke and/or a more severe stroke, or are at risk of developing vascular dementia.

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

Outputs from this work (positive or negative) will be disseminated to the scientific community through the publication of research and review articles, and through presentations at local, national, and international conferences. There are a number of clinicians working locally in this research area, which will allow us to investigate in humans

those new targets/concepts identified in our mouse studies. Also, any tissues collected from our studies will be made freely available for other researchers/collaborators.

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.

We will primarily use adult mice in our studies however some aspects of the work will be performed using aged mice (typically between 15-24 months of age) to better replicate the age that humans typically experience a stroke. Notably, ageing is well known to increase the chances of a person having a stroke, and strokes are often more severe in elderly humans. Therefore, it is important to also study processes and mechanisms in aged mice. We will use established models to induce stroke or vascular dementia in mice who have had genes deleted or mutated (and control mice who don't have these modifications) to enable us to accurately determine key processes involved in the diseases. These models of stroke or vascular dementia display many of the key disease features found in humans.

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

The majority of adult mice in this program of work will typically undergo 1 or 2 procedures prior to a stroke or vascular dementia being induced. These mice may have had a gene deleted or mutated. They may undergo procedures to characterise their baseline features which may include measuring blood pressure using a cuff device similar to that used on humans, treating the mice (e.g., via an injection, or in their drinking water) with a drug to target a specific pathway involved in the disease processes, or assessing their behaviours (e.g., memory, ability to perform tasks such as building a nest - mice will undergo up to 5 tests to assess their behaviour). If the mouse is deemed clinically healthy, stroke or vascular dementia will be induced under anaesthesia. Following recovery (and if deemed clinically healthy) they may undergo procedures to assess how their behaviours have changed after stroke or vascular dementia (mice will undergo up to 5 tests to assess their behaviour), and/or evaluating if a particular drug that targets a disease process can prevent or improve their behaviours. Also, some mice who have undergone stroke or vascular dementia induction, may also undergo a nonrecovery procedure under anaesthesia (e.g., Laser speckle contrast imaging which measures brain blood flow) to assess how stroke or vascular dementia affects blood flow in the brain. These experiments will last for 2-4 weeks.

To better replicate the age that humans typically experience a stroke, this program of work will also involve the study of mice between the ages of 15 and 24 months. If deemed clinically healthy, aged mice may undergo one procedure prior to stroke induction. Typically, an aged mouse will undergo up to 5 tests to assess their behaviours (e.g., memory and ability to perform tasks) or their blood pressure will be measured using a cuff device. Following stroke induction and if deemed clinically healthy, aged mice will typically undergo one procedure to assess how their behaviours have changed after stroke or vascular dementia. These experiments will last for 2-4 weeks.

To investigate the very rapid mechanisms that are triggered after stroke occurs, a small proportion of adult mice will undergo stroke induction under terminal anaesthesia. Under the same terminal anaesthesia, these mice may undergo a procedure to assess the rapid changes in brain blood flow after stroke induction.

To characterise the baseline features (i.e., in the absence of stroke or vascular dementia) a small proportion of adult mice may undergo multiple procedures (e.g., performing behaviour testing, taking blood samples, treatment with a drug, and then undergoing a non-recovery procedure under anaesthesia to assess blood flow in the brain). The mice used in these experiments may have had a gene deleted or mutated. Experiments may last for up to 16 weeks. During this period each mouse will typically undergo weekly blood pressure measurements (via a cuff device), plus up to 5 tests to assess their behaviours (e.g., memory and ability to perform tasks), and a single blood collection via an artery or vein.

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

All mice that undergo stroke or vascular dementia induction will experience brain damage and impaired motor and cognitive function (i.e., muscle weakness, un-coordination, impaired memory). Death from the surgery can occur. For example, up to 15% of mice may die because of stroke induction. It is predicted that all mice will experience weight loss following the induction of stroke or vascular dementia. Weight loss after stroke in humans is common and is believed to be caused by a disruption of normal metabolism due to the brain damage. The body weights of mice following stroke surgery typically stabilise at day 3. Mice are given a softened diet (rodent and baby food), hydration gel, and pre/post-surgery fluids via an injection to help maintaining their body weight. All mice will experience pain due to the incisions on their neck and head, although this is typically transient. Pain relief is given to all mice. Notably, stroke or vascular dementia themselves do not cause 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)?

Across all cohorts, we predict that approximately 10% of mice will undergo non-recovery procedures under anaesthesia; 30% of mice will undergo procedures that are sub-threshold/mild in severity; 30% will undergo procedures that are moderate in severity; and 30% will undergo procedures that are severe in severity.

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

- Killed

A retrospective assessment of these predicted harms will be due by 23 December 2028

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?

Brain blood vessels and the causes/consequences of stroke or vascular dementia are complex involving multiple cell types and tissues/organs. Therefore, to study these diseases we need to use a whole animal with a well-developed nervous, immune, and blood vessel systems. In this project we will use mouse disease models that have close similarities to stroke or vascular dementia in humans. Furthermore, there are many mouse strains available that have genes or proteins deleted or mutated, which will allow us to accurately identify the contribution of new pathways, mediators and processes involved in the diseases.

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

We have performed (and will continue to do so) internet searches to identify suitable alternative models, as well as using resources such as the NC3Rs website. However, the nature and complexity of stroke or vascular dementia means that non-animal alternatives cannot wholly replace the use of animals due to the complexity of brain blood vessels and the diseases. We have however developed cell-based models using cultured cells. We will continue to use these models in this program of work to further define mechanisms of brain blood vessel disease, and to test the efficacy of new drugs and their mechanism of action. Use of this model considerably reduces animal usage.

Why were they not suitable?

We have well characterised cell-based models using cultured cells, and where possible we replace the use of live animals with this model. Unfortunately, however, these models cannot fully replace the use of live animals due the complexity of brain blood vessels and disease mechanisms following stroke or during the development of vascular dementia. Indeed, brain blood vessels work together with other cells in the brain which is very challenging to recreate using just cells. Also, multiple cell types and organs are involved in the disease mechanisms after stroke or vascular dementia, and both diseases affect behaviour (e.g., muscle strength, memory and learning) which can only be studied in live animals.

A retrospective assessment of replacement will be due by 23 December 2028

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 mice has been estimated from our previous mouse usage in studies of this nature and by using calculations to predict the lowest number of mice required for each individual experiment.

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

We perform calculations to identify the lowest appropriate group sizes for each individual experiment. Also, we use resources such as the NC3Rs 'experimental design assistant' to ensure we are using the minimal number of animals possible to accurately answer our scientific questions. In the design of this project, we have adopted the quality standards required for clinical trials (e.g., randomisation, blinding, sample size calculations) and the recent IMPROVE recommendations, which were published to provide recommendations for improving the welfare of animals used for stroke research.

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

We closely monitor breeding efficiency to prevent the production of surplus mice and aim to use both sexes in our experiments. For example, we will use male and female mice to explore sex-dependent differences in disease mechanisms, and use female mice for baseline characterisation (e.g., verifying successful deletion of a gene of interest).
We use our cell-based models to test efficacy of new drugs before progressing to our mouse models. Together with calculations to predict the minimum number of mice required for an experiment, this prevents the unnecessary generation and use of live mice.
In all our studies, we bank various tissues/organs for future study thus preventing the unnecessary generation of additional mice. Furthermore, we make this tissue widely available to other researchers/collaborators which reduces the number of mice required across multiple projects.

A retrospective assessment of reduction will be due by 23 December 2028

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 carefully selected our models based on their similarities to the human condition. Importantly, mouse models display many of the key features of brain blood vessel disease found in humans (e.g., impaired ability of blood vessels to respond to stimuli, structural abnormalities). Similarly, although no stroke model is perfect, we have selected stroke models that closely resemble human stroke and those that cause the lowest possible degree of suffering (relative to other more severe models).

Our experiments have been designed to provide the maximum detailed characterisation whilst at the same time ensuring the animals under investigation experience the least pain, suffering, distress, and lasting harm. We will minimise pain, suffering, distress, and lasting harm by using stroke models that cause the lowest possible degree of suffering, through refinement control measures and using noninvasive imaging. These include the provision of pain relief medication, fluid support, soft foods, and a heat pad after surgeries. Furthermore, animals will be closely monitored and evaluated. If an animal's condition deteriorates, we will seek veterinary advice. We have developed clear humane criteria for euthanasia to limit potential suffering.

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

We need to use animals that have fully developed nervous, immune, and blood vessel systems to allow us to study the complex mechanisms that occur after a stroke or during vascular dementia. Mice represent the simplest sentient animal model available in which to investigate whole body mechanisms and consequences of such diseases. Some of the work can and will be performed on mice that are terminally anaesthetised; however, this approach does not allow us to wholly investigate the mechanisms and chronic consequences of these diseases on the brain and body.

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

Our experiments have been designed to provide the maximum detailed characterisation whilst at the same time ensuring the animals under investigation experience the least pain, suffering, distress, and lasting harm. We will minimise pain, suffering, distress, and lasting harm by using mouse models that cause the lowest possible degree of suffering, through refinement control measures, and using noninvasive imaging. These include the provision of pain relief medication, fluid support, softened foods, and a heat pad after surgeries. Furthermore, animals will be closely monitored and evaluated. If an animals' condition deteriorates, we will seek veterinary advice. We have developed monitoring sheets and clear humane criteria for euthanasia of both adult and aged mice to limit potential suffering.

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

For all our studies we follow ARRIVE guidelines to ensure good laboratory practice and transparent scientific reporting and use the IMPROVE guidelines relating to pre-clinical stroke studies.

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

We regularly visit the NC3Rs website to remain up to date with 3R resources, the publication of new methodologies and we adhere to ARRIVE and IMPROVE guidelines. Also, we will continue to attend our AWERB Culture of Care meetings which regularly meets to discuss topics pertaining to the 3Rs and the care and welfare of experimental animals at our establishment.

A retrospective assessment of refinement will be due by 23 December 2028

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?

19. Biological mechanisms of cardiovascular disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

cardiovascular disease, new therapies, blood vessels, heart, cells

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.

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 major aim of this Project is to identify new targets for the treatment of cardiovascular diseases by understanding common biology related to processes such as inflammation, metabolism and cell signalling.

A retrospective assessment of these aims will be due by 26 December 2028

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 primary goal of the project is to investigate the mechanisms which drive cardiovascular diseases such as heart failure, hypertension, and the causes of heart attacks atherosclerosis (blockages of blood vessels by fatty deposits). Cardiovascular disease is the largest cause of death in Western societies, and is as a major health problem in developing countries. New strategies to prevent or reverse cardiovascular disease are required to decrease deaths from this condition. In the last 20 years, much has been learnt about the mechanisms that regulate the health of the cardiovascular system. Although some of these treatments such as fat lowering therapies to treat atherosclerosis have been very successful, there are still a significant number of people for whom current treatments are not effective. Hence, there remains a pressing need to identify novel key factors in cardiovascular disease. It is only with the identification of novel pathways which alter the progression of cardiovascular disease that we will be able to develop new treatments for this condition.

It is now recognised that certain conditions such as diabetes worsen cardiovascular disease. In addition, other conditions such as the development high blood pressure during pregnancy (preeclampsia) have now been recognised to significantly increase the likelihood of both the mother and child developing cardiovascular disease later in life.

The mechanisms by which these comorbidities alter the development of cardiovascular disease is not well understood. This lack of understanding of the mechanisms underlying these conditions makes it very difficult to develop treatments for them. In this program of work, we will also investigate how our novel pathways alter cardiovascular disease development in the presence of these additional risk factors.

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

This project will advance our knowledge of factors which contribute to the initiation, progression and regression of cardiovascular diseases, and has the potential to identify new treatment targets for future prevention and treatment. Out puts will be:

- Publication of our results in scientific journals
- New information about cardiovascular disease
- The development of new treatments for cardiovascular disease

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

In the short-mid term 1-4 years:

We will present our results at scientific conferences to inform other scientist about our work as soon as possible

We will publish the results of our findings as soon as possible in scientific journals

Carry out public engagement to inform non-scientist about our work and why it is important

We will generate scientific resources which will be offered to other scientist to aid their research

Identify and communicate with other scientist from different areas of medicine e.g brain research who we think our work might be relevant to.

Long term - at the end of the project

Work with pharmaceutical company to test new treatments for cardiovascular disease

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

- Collaborate with other researchers and offer them reagents which have been developed during the course of our work
- Attend scientific meetings where we will discuss our work to raise awareness of our research
- Publish our work only in journals that are open access to ensure all researchers have access to our finding
- We will publish negative finding and also detailed descriptions of the methods we use including advancements and limitations
- Take part in public engagement work to raise awareness of cardiovascular disease its causes and how our research is helping to find new treatments

Species and numbers of animals expected to be used

- Mice: 33000

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.

Cardiovascular disease is a complex interplay between multiple factors in the body such as metabolic and inflammatory factors which act on different parts of the body such as the vasculature, nervous system and the heart to modify cardiovascular disease. Despite advancements in non animal alternatives such as computer modelling, cell based systems and data from clinical studies in patients with cardiovascular disease, these methods are still unable to fully model the complex biological processes in cardiovascular disease. The mouse has a very similar cardiovascular system and many of the process which lead to the formation of cardiovascular disease in human and mice are similar. Using specific experimental models in mice means we can mimic the type of cardiovascular diseases we observed in humans.

Most of the animals used in this license will be adult, as this is when cardiovascular disease is commonly found in humans. We will also use pregnant mice and the offspring of these pregnancies either before they are born or shortly after birth. This will enable us to look at how changes in the cardiovascular system impact on cardiovascular health of the mother and offspring during and after pregnancy. This is important as we do not understand why some mothers cardiovascular system does not adapt to pregnancy which leads to problems in cardiovascular function for both the mother and offspring later in life.

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

In order to answer the aims set out in this licence we will need to use genetically modified mice which model cardiovascular disease (e.g hypertension, atherosclerosis) or which

have alterations in the levels of genes which we think may have an important role in cardiovascular disease development. Genetically modified mice will be obtained from collaborators or commercial suppliers, in cases where mice are not available genetically modified mice will be generated by commercial suppliers. In some cases mice with cardiovascular disease will be bred with the genetically modified mice to enable use to look at the role of our gene of interest in cardiovascular disease. As is typical with humans it is expected that this will be asymptomatic. Some mice will be generated to model more severe forms of cardiovascular disease such as heart failure or alterations in blood vessels which may cause them to rupture.

We will use non-invasive imaging methods such as ultrasound and MRI to monitor disease progression. Some animals will receive multiple imaging sessions in order for us to monitor disease progression over a long period. We will also take small blood samples to monitor circulating factors which influence the cardiovascular system. In some case as with human animals may be fasted before this is done. In some cases we will administer substances which alter certain functions known to effect the progression of cardiovascular diseases such as drugs to alter the responses to inflammatory cells, or drugs which increase blood pressure. In order to mimic the cardiovascular problems associated with obesity and eating too much fat, diets which are high in fat and/or sugar may be given. As with humans exercise can be used to improve cardiovascular health caused by obesity and diabetes. To model this we put running wheels in cages to allowing animals to freely exercise so we can investigate the mechanism by which exercise improves cardiovascular health.

A small number of animals will undergo surgical procedures to allow us to investigate the common causes of cardiovascular disease. One of these models is thoracic aortic banding, a well-established procedure which narrows the aorta and is similar to the problems associated with aortic valve stenosis in humans. To generate this model an incision is made on the chest and a small suture is tied around the aorta to decrease the diameter which results in a gradual thickening of the heart wall. In another model we will tie off a coronary artery on the surface of the heart which will result in a heart attack. As before this is carried out by making an incision in the chest of the mouse. We will also use surgery to model cardiovascular surgical intervention used in humans such as bypass grafting and angioplasty to look at how genetic interventions alter the outcomes of current surgical treatments for cardiovascular disease. In these studies a vein or artery is grafted into another blood vessel or the vessel has an angioplasty wire inserted to mimic the damage cause by this intervention in humans. Animals will only have one surgery for a single cardiovascular disease model. We will use non-invasive imaging methods such as ultrasound and MRI to monitor disease progression. In some cases animals may be given substances by injection to treat cardiovascular disease. This will enable us to look at how our gene of interest effects recovery from cardiovascular disease. Animals will normally be humanely killed 1-4 weeks after surgery as this is enough time to allow us to look at how our gene of interest which we think plays a role in cardiovascular disease changes the initiation and/or progression of the disease.

In a very small number of studies, mice may have a combination of surgical models. In some mice, we may implant a device, which allows us to monitor the blood pressure and heart function of the mouse remotely 24h a day. Once this is implanted, the mouse is returned to its own cage. This enables us to carry out very detailed analysis of cardiovascular function in a mouse, which is not restrained and critically allows us to look at cardiovascular function when the mouse is asleep and awake. Once the mouse is fully recovered it may undergo a second surgery. For example implanted with a device which delivers a certain amount of drug throughout the day. This method means that we do not need to handle the mouse daily to administer the drug.

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

There are four severe protocols on this licence. However, it is anticipated that the vast majority of the animals on this licence will experience sub-threshold or mild procedures. Almost all genetically modified mice will have no adverse effects. A small proportion of mice could develop adverse effects such as lethargy and pain due to the development of heart failure. As we are interested in the processes that initiate cardiovascular disease we will aim to collect data from the majority of mice before these symptoms become apparent. The majority of procedure that we will carry out will only cause mild discomfort for a short period of time such as the recovery from a general anaesthetic to enable us to image the cardiovascular system, pain from an injection or from the collection of a small blood sample. If we need to give repeated injections or give drugs for longer periods of time we will implant a small drug delivery device under the skin. Most of the substances which we will give are not expected to cause adverse effect. Some drug may cause temporary discomfort for example drugs which change the way blood is distributed about the body can effect temperature control making animals hot. In this case we will give supportive measures such as providing mice with option of going to a cooler environment, these effects are transient and resolve after a few hours. Where possible we will try to monitor the cardiovascular system using methods which do not require the mouse to recover from surgery. For example, using non-invasive measurements where the mouse is gently restrained or alternatively taking these measurements under a terminal anaesthetic. In some cases where we need measurements for long periods of times or in un-restrained animals we may surgically implant devices which continuously measure cardiovascular function such as blood pressure. When we need to use surgical models animals are always given analgesia, are placed in a warm environment and monitored closely. In most cases animals recover full from the surgical procedure within 24h.

Mice will have treatments to induce cardiovascular disease such feeding animals a high fat diet to induce atherosclerosis. Feeding these diets as with humans can result in obesity and can cause the development of diabetes. Mice are closely monitored to make sure these circulating sugar levels do not get too high and that obesity does not affect the normal activities of the animal. Small number of animals will have surgery to induce cardiovascular disease e.g. heart failure by induction of a heart attack, or blockage of blood vessels to mimic the change in blood flow which cause fatty deposits to form in blood vessel. After surgery all animals are given analgesia, are placed in a warm environment and are monitored closely. In most cases animals recover from the surgical procedure within 48h. After surgery the major adverse effect that could occur is heart failure which results in weight loss, reduced mobility and breathlessness. The combination of close monitoring and sensitive imaging techniques means we can identify mice which are entering heart failure before any symptoms become apparent, enabling us to keep distress to a minimum. At the end of these experiments all of the animals will be humanely killed and tissue collected for biochemical and histological 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)?

Mice:
Sub-threshold 50%

Mild 20%
Moderate 20%
Severe 10%

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 26 December 2028

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?

Cardiovascular disease is a complex interplay between metabolic and inflammatory mechanisms acting in numerous systems such as the vasculature, nervous system and the heart. Despite advancements in computer modelling, In vitro cell based systems and the use of clinical studies in patients with cardiovascular disease, these methods are still unable to fully model the complex biological processes in cardiovascular disease. Hence the use of animals is unavoidable if important biological questions about this condition are to be addressed.

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

We use data from genetic studies in humans which help us to identify genes which have a role in cardiovascular disease. We also use data which has been produced by other laboratories and is publicly available this enables us to look at how the expression of our gene of interest changes in response to cardiovascular disease.

Where possible we use samples from people who have cardiovascular disease including samples of isolate inflammatory cells, vascular tissue (e.g. sample of atherosclerotic plaques and aneurysms), and cardiac myocytes.

We routinely use cell based assays to test the role of genes implicated in cardiovascular disease and potential therapeutic strategies in place of *in vivo* models. We have created cell based models that have been altered so we can change the expression of our genes of interest and we routinely utilize siRNA as a method to investigate consequence of loss of function of our genes of interest. Cell lines have been useful in establishing mechanism of action e.g. assays to establish interactions between inflammatory cells and endothelial cells.

Why were they not suitable?

Data from genetic studies in humans and publicly available data from human and animal models of cardiovascular disease is invaluable in helping us to understand the role of our gene of interest in cardiovascular disease but it does not enable us to investigate if our gene of interest causes cardiovascular disease or if it is a marker for disease.

Clinical samples enable us to investigate the relevance of cellular pathways and individual genes in cardiovascular disease tissue. However, these samples cannot tell us how important these mechanisms are for the initiation and progression of the disease.
Cell-based studies cannot replicate the complex interaction between different cell types and other factors (such as substances found in the blood) and so cannot address the impact of our manipulations on In vivo disease initiation, progression or regression.

A retrospective assessment of replacement will be due by 26 December 2028

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 use experiment conducted in our laboratory to enable us to accurately estimate how many animals will be required ensuring we can make statistical conclusions about our data. When we are carrying out an experiment for the first time we contact other laboratories to gather this information or we do extensive literature searches.
We design our experiments so we reduce animal numbers for example using a common control group for comparison between different drugs.

Where possible we aim to combine experiments so that multiple researchers can use tissue from the same experimental cohort for their own scientific means.

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

We frequently review relevant scientific literature to look for methods which will enable us to reduce animals usage.

We collaborate with other scientist to bring new methods to our laboratory which reduce animal usage We use website help with experimental design such as NC3R's Experimental Design Assistant (www.nc3rs.org.uk/experimental-design-assistant-eda) and PERPARE guidelines – (https://norecopa.no/prepare).

Our experiment comply with the ARRIVE guidelines (www.nc3rs.org.uk/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 will manage animal breeding following best practice in published guidelines to reduce animal numbers to the minimum required for our phenotyping experiments. We hold weekly lab meetings where we critically review animal usage including the estimated need for animals in the coming months. This enables us to ensure that animal over breeding is kept to a minimum. We work as a team to ensure that maximum use of all available tissue is made from each animal.

We invest in new technologies which require smaller sample sizes and enable us to derive more data from one sample. We have established new technologies which enable us to reduce the use of animals. We develop new methodologies so the data we generate has less variability which means that we require less animals to achieve statistical power. We have optimised protocols to allow multiple tissues from one mouse to be utilized for multiple assays for example for the isolation of primary vascular smooth muscle cells, macrophages and endothelial cells which means cells, can now be collected from multiple tissue sites allowing multiple researchers to utilize the tissue from one mouse.

A retrospective assessment of reduction will be due by 26 December 2028

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 disease models in this licence are already well established in our laboratory and we have worked hard to optimise animal welfare pre- and post-operatively. Detailed protocols have been written in collaboration with other groups who use these techniques in order to ensure best practice. This also includes frequent communications with collaborators and other scientists to establish if they have any refinements that would be applicable in our models.

We continue to develop new imaging techniques in rodents to increase sensitivity and decrease variability in our models. We are currently optimising a new imaging method (high resolution µCT) to image atherosclerosis. Traditional methods only give a 2D analysis of atherosclerosis, giving only a snap shot of atherosclerosis at a few anatomical locations. µCT enables us to image the volume of atherosclerosis in the whole of the aorta, decreasing the high variability associated with current quantification techniques. It also allows us to investigate location specific changes in atherosclerosis which allows us to look at how changes in blood flow patterns alters the development of atherosclerosis.

We are interested in the biology which causes cardiovascular disease as such we are most interested in what happens before animals develop symptoms of heart disease. We use non-invasive imaging such as ultrasound imaging of the cardiovascular system to monitor the progression of cardiovascular disease. This means the vast majority of our animals will never develop symptoms of cardiovascular disease.

As with humans certain strains of mice when feed a high fat diet will develop atherosclerosis. We frequently fed our mice a high fat diet to cause the development of atherosclerosis. Our extensive experience with the model enables us to feed mice for the minimal period of time to cause the required atherosclerotic plaque features. Unlike the diet the mice are normally given high fat diet is softer, hence mice are given additional chewing material to keep their teeth in good condition. In addition due to the greasy nature of the diet cages are changed more frequently and different types of bedding used to make sure the mice do not develop greasy coats.

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

Alternative animals such as Zebra fish have been considered and although these species have proven valuable in certain aspects of cardiovascular research such as angiogenesis and heart repair, they still have significant limitations as models of cardiovascular disease biology. For example, although Zebra fish can develop hyperlipidaemia at this time there are no Zebra fish models of atherosclerosis. Although Zebra fish are an excellent model system for heart regeneration post myocardial infarction this does not make them a suitable model for investigation the role of candidate genes in myocardial infarction pathology. In addition, although Zebra fish can model isolate pathologies associated with cardiovascular disease they cannot recapitulate the multi-factorial nature of cardiovascular disease.

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

We have many systems in place to minimise any suffering animal’s may experience during procedures. They are housed in a controlled environment with optimal lighting, heating, food and with appropriate companions. When we need to administer drugs or agents to mice we always pick the least invasive route, for instance in their food and drinking water or injections just under the skin. We also use the lowest effective dose when this is known. If we are using substances for the first time we carry out pilot tests on a small number of animals, always starting with the lowest dose and only increasing when necessary. Where possible we train animals for procedures to decrease stress levels during procedures. Anaesthesia and analgesia will be used for any procedures where the pain or discomfort could last longer than a few seconds. All surgery is carried out in a dedicated surgical room under sterile conditions. Animals are always given analgesia before and after surgery and are monitored closely after surgery.

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

www.nc3rs.org.uk https://norecopa.no https://www.lasa.co.uk

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

All scientist working under this licence attend frequent departmental meeting to discuss recent advances in 3Rs as well as attending establishment wide 3R meeting. In addition, scientists working under this licence join working groups for specific procedures which brings together scientist from multiple establishments to discuss advances in reduction and refinement methods. We will also consult our NC3Rs regional manager and the named information officer about any relevant advances in 3Rs.

We frequently search the literature to look for refinements to the techniques we use and consult relevant website such as www.nc3rs.org.uk https://science.rspca.org.uk

A retrospective assessment of refinement will be due by 26 December 2028

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?

20. Characterising tumour heterogeneity and differential therapeutic responses

Project duration

5 years 0 months

Project purpose

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

Key words

cancer, tumour heterogeneity, personalised therapy, biomarkers, tumour initiation, progression and metastasis

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.

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?

To characterise the diverse set of cells (heterogeneity) present in tumours to understand how cancer is formed and spread to distant organs.

We will study the above heterogeneity by classifying cancers into distinct subgroups (subtypes) based on their genetic variations and associate them with specific treatment responses in various cancer types that benefit the individual patient.

A retrospective assessment of these aims will be due by 27 December 2028

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?

Current standard-of-care therapies in the clinic for colorectal, pancreatic and breast cancers provide only modest benefits at the cost of significant side effects. Hence, there is a clear clinical need to tailor treatments and develop specialised diagnostic tools specific to patients based on the genetic variations of their tumours.

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

This project will generate various mouse models recapitulating patient tumours, which will help understand how tumours are formed, spread to distant organs, and respond to treatments. We anticipate these results to help initiate potential clinical trials to test novel therapies in patients. The research community will be informed of the outputs produced by this project through presentations at international scientific conferences and by publishing peer-reviewed publications.

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

Patients with aggressive subtypes of different cancer types with particularly poor outcomes respond less to standard-of-care therapies than other subtypes. Those who respond are at high risk of relapse and thus require further, often debilitating, treatment. In the short term (3 to 5 years), this project will help us to develop and validate diagnostic tools to select patients for particular therapies (for example, a subtype of colorectal cancer responds well to cetuximab (anti-epidermal growth factor receptor (antiEGFR) therapy). In the long term, the project is expected to enable clinicians, researchers, and pharmaceutical companies to test various drugs that may be subtype-specific, reduce therapy-related side effects, and prolong life.

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

The data generated from this project will be used as a springboard to clinically: a) develop diagnostic tools for early detection and sub-classification of various cancers; b) develop companion (associated with a drug) diagnostics for targeted therapies; and c)test promising drugs in preclinical animal tumour models and subsequent clinical trials. We will also develop additional collaborations with clinicians, bioengineers, bioinformaticians, and biologists and communicate our research at conferences and publish them in peer-reviewed journals.

Species and numbers of animals expected to be used

- Mice: 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.

Tumour involves the interaction of cancer cells with other cells (immune cells and cells related to scar tissues) within its microenvironment. It also interacts with different tissues or organs like lymph nodes and metastatic sites (a process of spreading to distant organs). Although cancer cells are cultured in the laboratory in vitro, these cultures do not recapitulate the cellular/tissue interactions. Specifically, tumour recurrence, metastasis, and responses to immunotherapies are impossible to mimic in vitro. Hence, mice are the most effective species for experiments requiring a recapitulation of patient tumours in vivo. Transplanted and genetically altered mice developing tumours are appropriate models to study cancer formation, spread to distant organs, cancer subtypes, tumour microenvironment and immunotherapy responses. For this project, we will use adult mice above 6 weeks old.

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

The genetically altered mice, which generate spontaneous tumours, will be generated by breeding appropriate strains of mice with genetic changes. Cancer cell transplanted mice models will be developed by injecting cancer cells or implanting tumour fragments subcutaneously, orthotopically (into relevant organs) and intravenously in mice. All these mice will be used to monitor tumour initiation, progression, and metastasis, by imaging or sacrificing them at the appropriate time of the investigation. Genetic function and/or therapeutic responses (after assessing optimal drug doses) will also be assessed using these mouse models.

At all times, mice will be kept in sterile cages with food, water, and clean bedding. Experience staff will undertake all the procedures to monitor the welfare of the mice.

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

Most mice will harbour tumour and/or metastasis at tolerable levels and equivalent clinical symptoms in humans. Aseptic techniques will be utilised throughout surgical treatments to prevent and reduce the possibility of wound infection. The mice may undergo weight loss, lethargy, and pain during various procedures, including surgery. General anesthesia and pre- and post-operative analgesics will be given to reduce any temporary pain or discomfort during surgical procedures and post-operative period. The mice may show responses to drugs similar to clinical symptoms in humans. However, if we have not tested these drugs in mice before, the potential side effects of the drugs are unknown. Therefore, we perform a small trial study in healthy mice to observe whether the drug can be well tolerated.

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 mice are expected to undergo mild to moderate categories. For example, breeding protocols involve mice with a 100% mild category. Those protocols that involve minor

surgery and carry a tumour will show a 99.5% moderate category. Rarely (0.05%) mice may experience a higher suffering because they respond adversely to a novel drug.

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

- Killed

A retrospective assessment of these predicted harms will be due by 27 December 2028

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?

Enormous progress has been made in cancer research by exploiting tumour cell lines that can be manipulated in vitro. However, there are a number of questions in cancer research that can only be addressed using animal models of disease. Firstly, cancer is a systemic (involving the whole body) disease involving interactions with multiple tissues during tumour initiation, progression, and metastasis. Secondly, it is challenging to recapitulate all complex interactions between the tumour and host cells in vitro.

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

Firstly, we apply computational methods to replace mice. The computational techniques mainly include a) grouping of patient tumours samples based on their similar genetic changes; b) identification of genetic information that distinguishes each cluster; and c) finally, finding a subset of samples that show response to certain therapies.
We also consider cancer cells from patients and mice that can be cultured in the laboratory (*in vitro*). We also co-culture cancer cells with immune cells. We strive to use these cultures in vitro as replacements for animals wherever we can. We are making every effort to establish better *in vitro* models that reflect the complex in vivo environment. Firstly, we perform the necessary experiments *in vitro*. We further conduct the experiments *in vivo* in mice only if necessary.

Why were they not suitable?

Because cancer cells interact with other cells in tumour, in vitro (laboratory culture of cells) models only provide a snapshot of changes in cancer cells. However, this does not contribute to the understanding tumour as a whole. Moreover, the interactions between cancer and immune cells cannot be effectively studied using in vitro models. Finally, it is challenging to recapitulate metastasis (cancer cells spreading to distant organs) in vitro. For these reasons, studies on in vivo tumour models in mice need to be performed, in which the benefits are weighted against the likely adverse effects and harms encountered.

A retrospective assessment of replacement will be due by 27 December 2028

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 aim of most of the studies (though certain studies may vary) is to: a) assess the effect of a drug on tumour volume over time compared to control or b) compare tumour volume between different subtype tumours. Since these tumour measurements will be taken from mice over time, methods for estimating the number of subjects required for longitudinal studies can also be employed here.

Also, we apply other computational methods per the requirement to reduce the number of mice. The computational techniques mainly include a) finding groups of tumours samples with similar genetic changes; b) identification of genetic information that distinguishes each cluster; and c) finally, finding a subset of samples that shows the response to certain therapies. Later, we perform cross-species analysis to select mouse models similar to each patient cluster and perform appropriate pre-clinical trials. This type of analysis has minimised the number of mice in the past and is expected to perform the same in future experiments.

We also have approximated the number of mice based on our prior research experience and statistical methodologies. Specifically, we applied statistics that helps to estimate the sample size required for an experiment to achieve statistically significant outcomes.

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

We involve the following strategies to minimise the number of animals.

Most of our experiments involve computational biology to validate our hypothesis initially. Later, we perform in vitro experiments to further test our hypothesis. For example, we have conducted multiple co-culture experiments to validate immune-based treatment effects in vitro. Based on the results from these two strategies, we perform optimal mouse experiments to study the changes systemically.

We involve those with statistical expertise to ensure that we use optimum group sizes and hence a minimum number of mice in our experiments.

We also designed mouse experiments based on our previous experience and literature evidence to reduce the number of experiments and mice.

We also use Experimental Design Assistant (EDA), a free online tool from NC3R, which helps design the experiment and helps reduce the number of mice according to the study objectives and experimental design.

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

We take a number of measures to ensure that the minimum number of mice is used. These include (a) using non-invasive imaging so that a single cohort of animals can be followed throughout an experiment without euthanising mice at various time points during the experiment, (b) we use the optimum procedures to reduce the number of mice. For example, every effort is made 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.

A retrospective assessment of reduction will be due by 27 December 2028

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 will be used in this project. Propagation of primary and metastatic tumours is a routine and refined procedure in cancer research using mice. This results in the generation of reproducible tumours in practical cohort sizes that are easy to monitor. The availability of genetically altered mouse strains that develop spontaneous tumours most closely mimic human tumour growth, cancer-immune interactions, tumour-related blood vessels, and response to treatment. Alternatively, we will inject cancer cells into mice to develop tumours that also recapitulate tumours developed in patients. Again, most of these genetically altered strains have been extensively studied in the cancer research community.

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

Previously, we performed a cross-species comparison of mouse and human tumour at the genetic level and found them to be similar. These genetic similarities between mouse and human tumours make mice suitable in vivo models for researching the process of tumour initiation, progression, and metastasis, and modelling tumour-stormal/immune interactions.

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 such as magnetic resonance imaging (MRI), but not limited to, monitoring tumour growth and the development of metastatic disease. The use of imaging modalities is to bring endpoints earlier, reduce harm, and minimise the suffering of the mice. For example, we inject cancer cells with a substance that glows (luminescence). These glowing cancer cells can be detected and quantitated via equipment. The more intense the luminescence, there is a large tumour. Hence, we end the experiment when the luminescence intensity exceeds a particular threshold and sacrifice the mice to take tumours out for further studies. This will enable us to stop the study earlier while still getting meaningful information and thus avoid unnecessary suffering.

Mice will be kept in sterilised bedding, food, and cages fitted with water supplies. During surgical operations, analgesia (to reduce pain) and anaesthesia (to eliminate sensation and pain) will be used to lessen stress and pain. Finally, suffering will be minimised by having experienced staff undertake all the procedures described.

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

Each team member is signed up to receive NC3R's publications and newsletter, which keeps them updated on any new information. We participate with internal forums which include the sharing of information on the care and welfare of laboratory animals.
We follow ARRIVE (Animal Research: Reporting of In Vivo Experiments), a checklist of recommendations to improve research reporting involving animals. We also use Experimental Design Assistant (EDA), a free online tool from NC3R, which helps design the experiment and helps reduce the number of mice according to the study objectives and experimental design.

A quarterly newsletter produced by our BSU will keep us updated on any new animal research information, including the 3Rs. To stay up to date on developments in the field, the entire team also goes to national or worldwide conferences.

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

When reporting our animal study, we adhere to the ARRIVE guidelines and NC3Rs resources to ensure sufficient detail is included. We also attend institutional Biological Service Unit's User's group meetings and read their newsletter regularly.

A retrospective assessment of refinement will be due by 27 December 2028

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?