Home Office

# Animals (Scientific Procedures) Act 1986

## Non-technical summaries for project licences granted January – June 2021 that require a retrospective assessment

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# 1. Development and Refinement of Methodology

**Project duration**

5 years 0 months

**Project purpose**

- 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**

Validation, Methodology, Refinement, Development

| Animal types | Life stages |
|---|---|
| Mice | juvenile, adult |
| Rats | juvenile, adult, pregnant |
| Rabbits | adult, pregnant |
| Minipigs | adult |
| Beagles | 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.

- Uses cats, dogs or equidae

## Objectives and benefits

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

**What's the aim of this project?**

The aim of this project is to develop new or refine current methodology for subsequent use on regulatory studies which assess the safety and efficacy of pharmaceutical, veterinary, agricultural and industrial chemicals which humans may be exposed to. Studies will also be performed to establish biological databases for animal models required where current data is not available or reliable e.g. may not have been generated using GLP principles, as well as supporting the validation and calibration of laboratory equipment used in a Good Laboratory Practice (GLP) environment.

**A Retrospective assessment of these aims will be due by 08 July 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

This licence has several objectives all of which will support regulatory studies being performed to GLP standards and in compliance with internationally accepted guidelines.

The decisions made for selecting a suitable species or strain of animal is primarily based on known biological data and the unique characteristics of that animal. In times where this data is either not available, or insufficient to enable scientific interpretation of data generated from their use, then studies can be performed to generate the biological databases necessary to allow scientific interpretation.

The success of regulatory studies i.e. achievement of their objectives, is very much reliant on the collection of accurate and reproducible data.

By developing and validating the methodology necessary to collect such data, including the validation and/or calibration of new or existing laboratory equipment used to generate or analyse data sets we can ensure that the data generated will not only permit scientific interpretation, but will also be accepted by regulatory bodies such as the FDA.

And finally, but not least, where there is scientific justification protected animals maybe used to assess the safety of a material before exposure to humans. It is essential therefore, that any procedure performed on them should cause the least amount of pain, suffering, distress or lasting harm practicable whilst achieving their objective. Studies performed under this licence will allow us to develop new techniques or refine existing ones thus, ensuring that the most up to date procedures are being used at all times and that animals are not exposed to procedures of higher severity than is necessary and do not experience undue pain, suffering, distress or lasting harm.

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

It is expected that at the end of this project procedures that are commonly performed on laboratory animals, for example, dose administration and blood sampling would have been refined to the extent that the levels of pain and or stress experienced are reduced, or the quality of samples (blood) produced are of greater quality or that analytical data can be acquired by the use of smaller sample volumes. Once Refinement has been fully validated the techniques can be applied to future regulatory studies performed at the Establishment, and where appropriate, shared with the scientific community via scientific papers, Industry Forums, presentations and/or seminars.

In addition to this, one of the primary outputs from this project is that future regulatory studies undertaken by the establishment will be performed using fully validated procedures and equipment as required by Good Laboratory Practice Standards, a prerequisite for regulatory submissions.

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

In the majority of cases the outputs of the studies performed i.e. laboratory equipment validated for use on regulatory studies and new methodology established for the performance of regulated procedures using animal models will be realised immediately. Initially, the advancements made will be used at the establishment, but it is likely that the Refinement of procedures performed on laboratory animals will be shared with scientific colleagues at other research establishments.

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

It is expected that the technical or Refinements developed from this work will not only be transferred to future regulatory studies performed at the Establishment,

but will also be shared with the scientific community via scientific papers, Industry Forums, presentations and symposiums.

**Species and numbers of animals expected to be used**

- Mice: 1000
- Rats: 1000
- Rabbits: 500
- Minipigs: 100
- Beagles: 100

## Predicted harms

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

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

The projects conducted under this licence will support regulatory studies which themselves use rats, mice, rabbits, minipig and the beagle dog and as such, these species will be used.

The majority of studies will use adult animals; However, where the studies are to support regulatory studies in juvenile animals then these will be used also.

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

The duration of the studies performed will be dependent on their objectives.

Typically, studies aimed at developing or refining techniques for blood sampling or the administration of a material for example, may take up to 28 days. As with most procedures, it is important to ensure that not only can they be repeated several times in a

single day (typically no more than 3 times in a 24 hour period), but repeatedly during the course of the study without causing undue discomfort, pain or distress to the animals. It is expected that no more than 20 animals will be required for this work.

Studies which assess the suitability of a rodent strain for use on long-term studies (life span) may be up to 104 weeks, the longest duration expected on regulatory Carcinogenicity studies. It is anticipated that up to 100 animals (50 males and 50 females) will be used on these study types. Studies using non- rodent species, e.g. the minipig do not usually need to be conducted for longer than 52 weeks.

Where the objective is to assess the survivability (lifespan) of the species/strain then the duration of the pilot study may be reduced or extended as appropriate.

The procedures performed may include the administration of a material using a route suitable for the species, the collection of blood and urine, behavioral tests and the collection of physiological data e.g. ECGs and blood pressure. Where animals are dosed using a route suitable for the species then dosing occasions will be no more than 3 occasions each day. Blood samples may be collected periodically from a suitable vein or artery, either to refine an existing blood sampling route or to provide samples to validate or calibrate laboratory equipment. Procedures will be performed in compliance with good practice guidelines.

Procedures will be performed the minimum frequency practicable to achieve the objectives of the study. Surgery will not be performed.

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

It is expected that the majority of animals used on this project will suffer no more than transient discomfort. It is possible that on rare occasions susceptible animals may exhibit short-term weight loss until they become accustomed to a particular procedure (typically no more than 5% of their body weight), but this would be considered exceptional and would only last a few days.

In some cases, particularly where the objective is to validate laboratory equipment which measures physiological responses for example, animals may be administered a positive control which brings about a particular physiological response; an increase in heart rate for example. Although not typically expected, it is possible that weight loss, or reduced weight gain may be observed for a few days (typically five or less), but this would be rare. A Reduction in food consumption may also be seen in some cases, but it is expected that normal appetite will return in less than five days.

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

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

The studies performed under this licence will be undertaken using either Mild or Moderate protocols. It is expected however, that 90% of all animals used will experience no more than Mild severity and 10% experiencing no more than short-term Moderate severity. Indeed, a review of statistics from similar work performed in a previous licence with the same objectives shows that 100% of all animals used experienced no more than Mild severity.

**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 08 July 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

The development of new techniques, for example novel ways for administering materials to animals, or the withdrawal of blood from non-typical veins can only be confirmed as being validated as suitable for repeated use without cause undue pain, suffering and distress to animals whilst achieving their purpose can only be achieved by performing them on live animals.

Similarly, the validation or calibration of some laboratory equipment needs tissue or biological samples from animals in order for them to be validated for subsequent use in a GLP environment and capable of producing accurate and consistent data. Whilst in some cases blood for example can be obtained from dead animals, it is not ethical to euthanize animals every time a sample is required; Live animals will therefore, be required for projects performed under this licence.

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

In very few cases the initial development or Refinement of a regulated procedure may be trialed using plastic models of animals or replicas of body parts.

**Why were they not suitable?**

Studies using plastic animal models for example cannot accurately demonstrate the difficulties a procedure may present when performed on a live animals; or any undue pain, suffering or distress

that may be caused during its performance.

**A Retrospective assessment of Replacement will be due by 08 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

The numbers of animals potentially being used have been estimated by considering the ratio of studies which evaluate the Refinement of regulatory procedures, those which establish method development and those which ascertain the suitability of a species for use on regulatory studies (potentially the largest user of animals) and the number of animals required for each study type. Consideration has also been given to animal usage on previous licences with similar objectives.

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

There are no relevant regulatory requirements for the studies being performed under this licence and as such, there are no guidance documents on the numbers of animals that should be used. The numbers of animals used will therefore, be assessed on a case by case basis.

Where relevant, we will review the data generated from previous studies performed under the authority of previous licences with similar scope and objectives. This data generated will provide us with refined study plans and typical numbers of animals required in order to achieve our objectives.

In the majority of cases studies will utilise animals of both sexes to ensure that the relevant data generated fully supports future regulatory studies which typically use both sexes; but consideration will always be given to whether the objectives of the study can be achieved by the use of a single sex and will not affect the integrity of regulatory studies they aim to support. Examples of such objectives may include the Refinement of a blood sampling technique whereby, performance of the procedure and successful outcome is not prohibited by the sex of the animal.

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

The studies performed under this licence will be similar to those performed on previous licences. We will look at the numbers of animals used for each particular purpose and evaluate if the numbers used can be reduced in any way.

**A Retrospective assessment of Reduction will be due by 08 July 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

The primary objective of this licence is to develop new techniques (primarily dose administration or the collection of body fluids e.g. blood) for use on common laboratory animals which are to be used on subsequent regulatory efficacy and safety studies required by legislative bodies. The animal models used will be rats, mice, rabbits, minipigs and dogs.

The methods and techniques used will be adaptations of current techniques, or Refinement of current ones.

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

In order to suitably validate the success of a technique it needs to be developed using animals of the same age, or at least size that the techniques will be performed on. For example, administering a substance by oral gavage to a juvenile rat requires different equipment and technique to an adult rat. In many cases, techniques are not transferable between stages of development or indeed species.

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

The purpose of this licence is to refine procedures with the intention of reducing levels of undue, pain, suffering and distress during their performance. The procedures themselves will not require surgery, or expected to cause more than transient discomfort. All animals will be routinely observed a minimum of twice each day for a suitable duration (dependent on the duration of the study) by experienced Personal licence holders familiar with the species to ensure that no adverse reactions detrimental to the health of the animals occur.

In the unlikely event of an animal being injured or showing unexpected signs of ill health it will be humanely killed.

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

J. Applied Toxicol. 21, 15-23 (2001); A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Karl-Heinz Diehl et-al.

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

Our establishment has a number of personal licence holders who are members of recognised Forums that discuss topics relating to animal welfare and Refinement of procedures. Topics of interest are disseminated to interested parties, including our Animal Welfare and Ethical Review Body on which I am a member, by participants of the Forum and/or the Named Information Officer.

In addition to this, we periodically search for advances in the 3R's via appropriate websites such as The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

**A Retrospective assessment of Refinement will be due by 08 July 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?

# 2. Colony Management, Production & Preservation of GA animals as a service & associated support

**Project duration**

5 years 0 months

**Project purpose**

- Basic research

**Key words**

colony management, cryopreservation, GA production, rederivation, Monoclonal Antibodies

| Animal types | Life stages |
|---|---|
| Mice | adult, juvenile, embryo, neonate, pregnant |
| Zebra fish | embryo, neonate, juvenile, adult |
| Xenopus laevis | embryo, 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.

- 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 provide centrally managed breeding colonies of Genetically Altered (GA) and Wild-type (Wt) mice, zebrafish & xenopus, and associated procedures such as new GA line production at a high and consistent standard to the scientific community at this Institute.

**A Retrospective assessment of these aims will be due by 11 July 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 accr**ue **after the project has finished.**

**Why is it important to undertake this work?**

The Establishment holds multiple genetically altered mouse, zebrafish and Xenopus colonies live at any one time for the diverse portfolio of work undertaken in the field of medical research. There are ~700 new lines created or imported each year and a similar number are archived off the shelf or for distribution to collaborators. Providing this service centrally and in a demand-matched breeding mode, supported and managed by specialised technical experts is administratively efficient and has proved welfare benefits.

Our main outputs will be

- Centralisation of common use lines ensuring minimisation of waste as well as best standards of care
- Production of sophisticated and refined GA models for the research here,
- A Cryopreservation service to archive rodent & aquatic species, ensuring lines are backed up and animal numbers are reduced,
- Rederivation of all imported lines to ensure infection free mice at the highest health status, allowing distribution between barriered facilities, and allowing stability & characterisation of the microbial population, reducing phenotypic & scientific variation.

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

This is a service licence and as such we expect to have maintained our colonies of mice and fish in an efficient manner, to have exceeded best practice in colony management, to have created ~100 novel sophisticated GA lines and maintained them at the highest health status. To have cryopreserved >2000 lines, and rederived all lines entering the establishment the highest health status.

To support us in this we have a team whose focus is on implementing best practice and analysing breeding across the establishment to enable us to exceed our performance year on year. For example, we have a comprehensive mouse database and analyse the data through Power BI reports looking at PEI, mortality rates, trios v pairs, mating age etc.

We also expect to have worked to refine the techniques we use, such as optimising surgical techniques, taken on new technologies to make our work more efficient and enable us to reduce animal numbers. To have trained staff and scientists in best practice, and shared that through the use of journals, presentations and technical forums. We look to places like the NC3RS for best practice or to share our own findings for improved outcomes.

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

Providing this service centrally and in a demand-matched breeding mode, supported and managed by specialised technical experts is administratively efficient and has proven welfare benefits. The protocols covered by this licence will benefit animal users across the

establishment, all of whom will have their own PPLs with benefits clearly described in the field of medical research. We have a policy where we don't allow duplicates of any colony, and keep all colonies at the highest health status. As a consequence, we can minimise waste and use spare stock for cryopreservation. We also encourage use of cryopreserved stock to manage a line, many Cre lines for example can be maintained from the freezer, and then an IVF carried out by our skilled transgenic team as and when the line is needed, minimising overall animal use and waste.

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

We will disseminate best practice through training courses, journals, presentations and technical forums.

**Species and numbers of animals expected to be used**

- Mice: 350,000
- Xenopus laevis: 2000
- Zebra fish (Danio rerio): 45,000

## Predicted harms

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

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

This is a service licence for the establishment for mouse, zebrafish and Xenopus users. By making these services available centrally we can ensure reduced numbers of animals and best practice in welfare. These animals & life stages are the most appropriate for the breeding and production of GA animals.

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

Most of the animals used on this licence will be of a mild severity and be bred for use in other experimental project licences.

Where new GA lines are produced, cryopreserved or rederived, an animal might undergo superovulation - a mild procedure involving an injection with hormones on 2 occasions causing it to produce a higher number of embryos than is normal before being mated and killed. A very small number (<5%) might undergo a repeat procedure if they failed to mate, and the NVS deemed the animals' welfare to allow it.

Alternatively, an animal might be mated with a sterile male to render it "pseudopregnant" and then undergo a moderate surgical procedure of 5- 10 minutes under analgesia and anaesthesia whereby genetically altered embryos are implanted. The mouse will then carry to term as normal and give birth to a litter of GA mice.

Zebrafish generally breed naturally, or a small percentage might be used for a procedure whereby their eggs are collected under analgesia and anaesthetic (administered in their water) by gently but firmly stroking the belly before putting them back to recover.

Xenopus normally would lay naturally, but on occasion for collection of sperm may undergo a hormone injection and Females also undergo superovulation to produce more eggs than they would naturally.

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

Most animals in this PPL are not expected to undergo more than transient discomfort. In the case of surgery, the animals will experience mild pain, are expected to make a rapid and unremarkable recovery. Animals will be monitored daily and in the event of post-operative complications, animals will be killed unless, in the opinion of the Named Veterinary Surgeon, such complications can be remedied promptly and successfully using no more than minor interventions. Analgesic agents will be administered as 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)?**

~70% of mice are expected to be subthreshold and 17% mild, 6%of mice are expected to be moderate (surgical) and 6% of mice are expected to be severe - because they were found dead with no prior clinical signs

~74% of fish experience mild severity, 23% severe and 2 % moderate.

~97% of Xenopus experience mild severity

**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 11 July 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

This is a service licence to produce and maintain genetically altered animals and aquatic species to the Science here. All Projects using our animals will justify their work in detail with regards to medical research.

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

N/A

**Why were they not suitable?**

N/A

**A Retrospective assessment of Replacement will be due by 11 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

**How have you estimated the numbers of animals you will use?**
The numbers estimated here are based on the likely level of work we will experience, knowing the data from the last 5 years. Demand is reasonably stable, and the Institute has a regular supply of new animal users who will use this service.
The number of animals used in each protocol is based on experience and we are constantly working to reduce those numbers. For example, in the production of GA animals, we know how strains will respond to hormone treatment and how many embryos we can get from each animal. We also know the average success rates of different procedures and ensure our staff are trained to maintain and improve on those numbers. An example would be we use ~10 mice treated with hormones to collect in excess of 300 embryos. These are genetically manipulated and will be transferred into ~10 females, and ~40 pups will be born. We have recently refined this process to use hyperovulation where possible, which in some strains ~doubles the number of eggs collected, halving the number of animals we use.

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

This has all been done on experience, working to best practice guidelines in the field and maintaining those high standards. These are reviewed by our AWERB procedures regularly.

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

The number of animals used in the pursuit of this PPL's objectives will be kept to a minimum through training of staff to high standards, and monitoring success rates, such

that benchmarks are set for staff to improve on. We have an excellent mouse colony management database, which is already allowing us much better control in tracking our animal usage, health concerns, the genetic background and modifications of our mice, and any phenotypic concerns. we have detailed Power BI reports that look at multiple aspects of our breeding and animal use. This allows us to both refine the husbandry and care of our animals and keep numbers to a minimum – the allele tracking has already allowed us to identify 10s of colonies where there were duplication, and savings have been made.

Our databases keep track of all our work, monitor the embryo output of strains, success rates of constructs and ES cells, transgenic and embryo transfer rates, allowing us to recommend best practice, put limits on animal use and spot problems as they occur and investigate them.

We have a new breeding optimisation service who coordinate this effort and also work with all groups using animals to refine & optimise breeding. Animals are only bred if user requirement has been established, and the breeding programme is subject to regular review to optimally meet anticipated demand. Spare animals are made available for use on other scientific projects. Colony plans and breeding schemes are regularly reviewed to ensure minimal wastage.
Unnecessary production or import of GAA is avoided by searching cryobanks and databases. Examples of resources available include: NC3R's mouse database, Animal Welfare Management Discussion Group (AWMDG), Mouse locator, PubMed, the Jackson laboratory, and various Cre databases.

Cryopreservation helps to reduce animal numbers removing strains no longer in use and allowing maintenance via smaller live colonies. We track cryopreservation survival rates and IVF fertilisation rates so we can use the most appropriate route for each strain, keeping animal numbers to a minimum. Cryopreservation of rodent strains is relatively common, but by implementing a service for preserving frogs and fish as well, we can extend the advantages to these species as well.

By centralising the provision of animals, we can provide a demand matched supply and reduce wastage. We can use any spare animals to cryopreserve embryos for our GAA production service, or for the production of rodent serum. We use datasheets, genetic monitoring and microbiome monitoring to enable us to track our animals in as many ways as possible to manage any harm to them or possible change to their phenotypes – thus allowing us to keep numbers to a minimum and welfare high.

**A Retrospective assessment of Reduction will be due by 11 July 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

**procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging Refinement techniques during the lifetime of the project.**

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

We use mice, zebrafish and Xenopus in this project.

All communally bred mice bred under the PPL are bred under the mild protocol baring one strain (severe). Occasionally there will be need for the moderate protocol when importing new lines. The methods we use in the production of new GA animals are all refined to reduce suffering to a minimum. The Zebrafish and Xenopus models are also mild.

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

This service PPL by its nature requires the use of animals and will result in GAA being made available for use in most of the PPLs used at the establishment, for which the benefits are clearly described within each PPL and will be published via the scientific groups holding these PPLs. Much of the work covered here requires further justification through an application process, and if it is felt that alternatives are available, the work will be turned down.

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

We make every effort to refine our procedures wherever possible, and there is a definite culture of care within the service with many animal care and transgenic technicians presenting on 3Rs work at animal welfare meetings.

We continue to instigate many changes to our Surgical techniques, taking tips from the LASA Surgical technical forums and from the LASA recommendations for aseptic surgery and have worked closely with the vet to ensure our pre, peri and post-operative care is the best we can offer. We have extensively used non-surgical embryos transfer over the past 5 years, particularly in our rederivation work. There are still higher rates of success for surgical, and both methods have their advantages and disadvantages and are used dependent on the stage and manipulation applied to the embryo.

Breeding is mild for the most part by keeping lines with moderate or severe phenotypes breeding heterozgously wherever possible, and using them before a phenotype appears – but there will be occasions where for experimental need, the homozygous line needs to be maintained.

Cryopreservation is encouraged, as a means of reducing the welfare issues involved in animal shipment as well as to take animals off the shelf and reduce waste, moving towards managing colonies from the freezer. We also freeze a very high volume of lines through sperm cryopreservation, reducing the procedures needed using animals to archive a strain.

We keep up to date of new genetic tools be they new gene editing techniques such as endonucleases or novel inducible, conditional, spatial specific and binary systems which reduce the severity of phenotypes in GAA to pass on to users and to update the general use lines we have available. Our transgenic rates are assessed and improved upon through a variety of minor refinements and come in above average in an international survey of transgenic services, and have contributed to a large range of research.

New environmental enrichment products are trialled continuously by animal care staff, and there is an active training and development programme for animal care staff ensuring best practice.

We use ear clips as standard for genotyping, and are working with a collaborator on faecal samples for repeat sampling and ensure all our staff are trained in best practice and constantly search for methods to refine our techniques. New GA mice undergo welfare assessment, and passports are used where mice are transported to ensure continued high standards of welfare. Our mice undergo a genetic stability program to ensure we know the background and regularly refresh our lines to minimise drift and phenotypic variation. We also monitor the microbiome for that purpose.

With regards to antibody work we always choose the least severe method, and keep abreast of new adjuvants to minimise side effects.

We rarely use vasectomy since we moved to a strain with sterile males.

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

We work with the LASA guidelines for Aseptic Surgical technique, NC3RS and RSPCA guidelines in the transport of animals, maintenance and production of GA animals, cryopreservation & archiving as well as those published by expert working groups and published in Lab Animals (such as those on health monitoring and gene editing)

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

Our staff all participate actively in improving their skills sets and receive mandatory Home Office training, IAT qualifications from our onsite training team as well as attending local inductions, refresher and best practice sessions.

We have regular attendance at seminars workshops and conferences in the field of Lab animal science, colony management and GA production & maintenance. These are reported back to a wider group through our animal technical forums as well as through the department seminar series, the NIO's newsletter and throughout technician training team. keeping abreast of advances through journals, email discussion groups and technical forums as well as the useful NC3RS technician hub and GA resources ensures we are at the forefront of new advances in the 3Rs.

**A Retrospective assessment of Refinement will be due by 11 July 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?

# 3. Central nervous system regeneration and repair in zebrafish

**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**

spinal cord injury, live observation, neurodegenerative disease, progenitor cells, axons

| Animal types | Life stages |
|---|---|
| Zebra fish | pregnant, adult, juvenile, embryo, neonate, aged |

## Retrospective assessment

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

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?**

Zebrafish, in contrast to humans, can repair damage to their central nervous system. We want to determine how these vertebrates can achieve Replacement of lost nerve cells and reestablishment of injured connections in order to inform future therapeutic efforts in non-regenerating vertebrates.

**A Retrospective assessment of these aims will be due by 25 July 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Any loss of nervous tissue in humans, be it after acute injury, such as stroke or spinal cord injury, or neurodegenerative disorders, such as Parkinsons's disease, leads to mostly irreparable loss of nerve cells and their connections, which entails life-long debilitating consequences. Because of the general lack of repair in complex injury sites, there is uncertainty about the most important parameters that need to be targeted in order to achieve repair. In the zebrafish, we are presented with a vertebrate that shows Replacement of nerve cells and regrowth of nerve connections. At the same time, the zebrafish is a highly accessible experimental animal, that allows relatively easy creation of genetic models and, at the larval stage, direct observation of regeneration. Hence, we can unravel the cell types and molecular mechanism in zebrafish to gain fundamental insight into successful central nervous system repair in a vertebrate and inform future therapies in non-regenerating mammals.

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

Zebrafish, in contrast to humans, have the capacity to repair their central nervous system after injury. The outputs for this project will primarily be scientific insights into how zebrafish accomplish repair by replacing lost nerve cells and their connections. We will identify critical cell types and molecular signals that govern successful regeneration. Moreover, we will use screening assays in zebrafish to identify drugs and genes that are essential for regeneration. This will provide starting points and targets for potential therapeutic approaches in non-regenerating mammalian models. We will present our results at national and international scientific conferences and publish in scientific journals. Moreover, we expect to train several PhD students and post-doctoral researchers in regeneration research.

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

In the short term, our results will benefit the scientific community by improving our understanding of the mechanisms of central nervous system regeneration.

In particular our screening approaches aim to immediately inform our collaborators who work on mammalian regeneration system, for example of drugs that can control the inflammatory response to central nervous system injury. Ultimately, our findings aim to contribute to future therapeutic approaches to devasting human conditions, such as spinal cord injury. There is currently no treatment for this condition and patients have to live with life- long disabilities.

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

We will maximise the impact of our findings through collaborations with groups that work on central nervous system injury, who are already working with us in scientific consortia. In

this way, promising drugs or drug-targets that we discover in zebrafish will go into trials in mammalian model systems without delay. Moreover, we will present our results at national and international scientific meetings, and in scientific journals that are highly visible in the scientific community. We will also communicate our results to the wider public. The PI and postgraduates have and will continue to run engagement events for local schools, host high school pupils, and the groups' postgraduates have and will run engagement events (e.g. at science fairs).

**Species and numbers of animals expected to be used**

- Zebra fish (Danio rerio): 35.000 (larval) 1000 (adult)

## 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**.

Injuries to or degenerative diseases of the brain and spinal cord in humans are hardly repaired, leading to permanent disabilities, such as life-long paralysis after spinal cord injury or in Parkinson's disease.

Our model organism, the zebrafish, repairs nervous tissue without any intervention. This offers the opportunity to learn how repair is accomplished in the brain and spinal cord in a vertebrate. We focus mostly on larval zebrafish because at that stage repair is very rapid and larvae are still transparent, such that we can directly observe regenerative processes by video-microscopy. However, in some instances it is important to analyse regenerative processes in adult animals, because in larval animals not all cell types are present that could influence the repair outcome and larger dimensions of adult animals could reveal additional repair mechanisms that are necessary to bridge larger distances in

repair. In this way, we will identify important cells and molecular signals that will serve to develop therapeutic targets in non-regenerating mammals.

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

Typically, zebrafish will be deeply anaesthetised and receive an injury to their brain or spinal cord. We will either injur the tissue by inserting a needle into the brain or severing the spinal cord. We may also use drugs to remove only specific cells, such as dopaminergic neurons to mimic the specific conditions, such as Parkinson's Disease. Importantly, most of these injuries will take place at early larval stages before animals are protected by law, because the larval nervous system is not sufficiently developed to process pain. We will then observe how the tissue is repaired using video-microscopy of specific cell types, such as immune cells, nerve cells, and stem cells in the nervous system and by testing how quickly animals regain swimming function, e.g. after spinal cord injury. Using state-of-the-art genetic techniques, we will manipulate the activity of specific cells and genes to determine whether this changes the repair process. This in turn will

allow us to draw conclusions as to the relative importance of these cells and genes for successful brain and spinal cord repair in zebrafish and their likely relevance on non-regenerating species.

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

For larvae that are injured before they become protected, their injury will be mostly healed when they are sufficiently developed to be protected, such that we do not expect major harm to these larvae. If an experimental manipulation leads to delayed healing, experiments can often be concluded before larvae become protected. In some cases larvae may have to be kept longer and they may experience reduced swimming capacity or abnormal capacity to swim. We will compensate for that by giving larvae additional food. These longer observation periods may take several days after they become protected at 5 days of development. Post-larval and adult animals may also undergo the above types of injury. In our experience (> 20 years) animals wake up from anaesthesia within minutes after surgery and take food immediately. After removal of specific cell types, behaviours are almost completely normal, but there are some subtle swimming abnormalities that do not prevent them from feeding. After a spinal cord injury, animals are paralysed, but can move with the help of their pectoral fins and also feed directly after surgery. They regain full swimming capacity within six weeks after injury.

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

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

We will generate and breed genetically altered zebrafish in the majority of procedures. These manipulations are to highlight specific cell types with fluorescent proteins or pertain to genes that may play a role in regeneration and do not cause gross abnormalities on their own. Therefore, we expect that > 90% of animals do not experience any adverse effect from their genetic alterations per se. In larval lesions, most experiments will be analysed before larvae become protected and deficits will be subtle for those larve that are kept for longer observations, such that > 90% of larvae will not experience even moderate symptoms. Adult fish will be deeply anaesthetised for surgery. As they come out of anaesthesia, we cannot use analgaesics, because these are likely to interfere with the very processes we are studying. As we cannot exclude post-operative pain, all adult injuries are categorised as severe procedures. However, fish immediately right themselves and readily feed on live artemia after surgery, suggesting that they cope well with the procedures. Based on our experience, we have refined post- operative care in these animals, including individual feeding, anti-bacterial medication, and seclusion during the initial post-operative days to ensure optimal recovery.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 25 July 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?**

Central nervous system injuries are highly complex, involving a number of interacting cell types, such as different types of immune cells and nerve cells. Moreover, these interactions change over time, e.g. when the initial inflammatory response is suppressed by later arriving cell types. These interactions determine regenerative success and must therefore be analysed together.

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

We are successfully using two types of alternatives to the use of protected animals wherever possible.

We use larvae at unprotected early stages, up to 5 days post fertilisation, when their capacity to feel pain is not sufficiently developed.

We use isolated progenitor cells from fish in an ex vivo preparation to study the reaction of these cells to specific signals.

**Why were they not suitable?**

We have to use larvae at protected stages when repair processes are protracted and carry an animal into the protected period after initial injury. For example, some new nerve cells may need days to acquire their final state. Moreover, if effects of manipulations are observed at unprotected stages, we need to determine if these are transient or later repaired to find potential compensatory mechanisms.

Finally, some experiments need to be done on adult animals, because these possess an adaptive immune system that has decisive influence on regeneration, but is not sufficiently developed in larvae. Moreover, dimension in adult are larger, potentially requiring additional mechanisms of regeneration that are not needed at larval stages.

Using ex vivo preparations is very useful when a specific signalling molecule is to be tested, but this assay lacks the complexity of the in vivo system to identify unknown cell interactions.

In general, cell culture experiments complement in vivo research and will be used wherever possible, but they fail to reproduce the in vivo complexity, such that presently we cannot completely avoid the use of animals, yet.

**A Retrospective assessment of Replacement will be due by 25 July 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?**

Most animals we use are necessary for breeding of genetically altered fish that we use to indicate cell types or manipulate specific cell types or molecules. The vast majority of animals will not experience more than mild phenotypes. The number of animals we will need for breeding purposes are based on our (>20 years) experience with breeding and generating such animals. In our experimental protocols, we strive to use the minimal number of animals to be able to make robust scientific statements that stand up to peer-review. Again, our estimates are informed by the number of animals needed in previous similar experiments.

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

In our experimental design phase we observe best practice to reduce the number of animals used. For example, we reduce experimental bias by randomisation of animals into experimental groups, blinding of the experimental condition to the observer, clear determination of experimental units, assignment of only the necessary control groups and sample size calculations to arrive at the minimal number of animals needed to make a sufficiently robust observation.

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

For maintenance of transgenic fish lines, we will only use the minimal number of individuals necessary to propagate the lines and have sufficient larvae available for experiments. Generating genetically altered fish involves injection of genetic material into eggs that will be integrated into the genome in only a fraction of the animals. We will use fluorescent reporter constructs to be able to see successful recombination before the larvae become protected, such that we do not need to let unsuccessfully manipulated

individuals enter the protected period. Moreover, we are using highly efficient means of genetic manipulations, which means we need to inject fewer eggs, reducing the number of animals needed.

In our experimental protocols we will use, wherever possible, animals that allow more than one read- out. For example, we can generate double-transgenic animals that allow us to observe the reaction of neurons and non-neural cells in the same animals, reducing the overall number of animals needed to study these cell types.

Moreover, we will use power calculations to determine the optimal number of animals needed. Where the experimental variability is not known, because an experimental design is new, we will use pilot studies with few individuals to obtain a basis for power calculations.

**A Retrospective assessment of Reduction will be due by 25 July 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.**

Most of our experiments will be done on larvae at pre-feeding stages, when their nervous system is insufficiently developed to feel pain. Consequently, these animals are not protected by the ASPA. Larvae will receive an injury to their spinal cord or brain under anaesthesia and we will use optical methods on these transparent larvae to observe the rapid regeneration of the nervous tissue. We have developed this model during the last project licence period and this has reduced our need to use protected animals significantly.

However, in some cases regeneration takes larvae into the protected period. Moreover, we still need to use a low proportion of adult animals to study features, that are not present in larvae, such as cell of the adaptive immune system that are important for regeneration.

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

We are studying successful central nervous system regeneration, which can be observed in salamanders and fishes. Arguably, fish, particularly larval forms, present vertebrates of

relatively low sentience compared to mammals. We make sure that most experiments are done under terminal anaesthesia, such as imaging of cell movements after injury. However, after the initial injury, fish need to be revived to allow time for regeneration to take place.

Animals of even lower sentience, such as insects or worms, are unsuitable for our research, because they show strong genetic and anatomical divergence from vertebrates that would jeopardise translation of our findings to mammalian systems.

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

We will strive to use as few animals at protected stages as possible. Over the previous project period we have improved monitoring schemes for experiments on protected stages and we are striving to improve post-operative care, for example by keeping animals behind a tarp to reduce unnecessary startle responses. We control their water conditions and ambient temperature and individually feed experimental fish with live feed from a pipette.

These measures have been developed by us and strongly improve recovery of fish after surgery. We are teaching these to other laboratories world- wide.

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

We will observe best practice in our experiments that is in part developed by ourselves and will incorporate any new welfare steps that are introduced by the NC3Rs or the latest FELASA guidelines on zebrafish use (http://www.felasa.eu/working-groups/working-groups-present/severity-classification- in-zebrafish/)

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

We will at all stages during the project receive the latest guidance through the local NVS, the NC3R newsletter and discussions with colleagues, FELASA guidelines on zebrafish use (http://www.felasa.eu/working-groups/working-groups-present/severity-classification-in-zebrafish/)

**A Retrospective assessment of Refinement will be due by 25 July 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?

# 4. Role of nitric oxide and reactive oxygen species in cardiac function

**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**

Heart, Nitric Oxide, Reactive Oxygen Species, Cardiac Remodelling, Cardiac Disease

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

## Retrospective assessment

The Secretary of State has determined that a Retrospective assessment of this licence is 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 role of nitric oxide (NO) and reactive oxygen species (ROS) signalling pathways in the pathogenesis of common cardiovascular diseases, such as atrial fibrillation (AF) and heart failure with preserved left ventricular ejection fraction (HFpEF), with the ultimate objective of identifying novel druggable targets.

**A Retrospective assessment of these aims will be due by 22 July 2026**

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

**Did the project achieve it's aims and if not, why not?**

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

**Why is it important to undertake this work?**

Notwithstanding the major disease burden of AF and HFpEF across the world, there are currently no therapies improving the likely course of these medical conditions (other than anticoagulation to prevent the thromboembolic complications of AF). Our previous work has identified important roles for NO and ROS signalling in human disease and promising pathways that need to be further tested by using a combination of genetic tools and genetically modified animal models.

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

We will be studying the role of different molecules (e.g. nitric oxide and reactive oxygen species) in the healthy and diseased heart and identifying new ways in which the levels of these molecules can be modulated in our hearts. Together with the identification of new biomarkers of disease evolution we will aim to restore normal myocardial function or retard the evolution towards heart failure and atrial fibrillation in chronically stressed hearts.

These results will be published in international peer-reviewed scientific journals and presented in scientific conferences.

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

Most benefits from these outputs will be seen in the long term. It is likely that our results will improve the understanding of how nitric oxide and reactive oxygen species regulate cardiac function and that this knowledge will guide future investigations:

Our data collected from isolated cardiomyocytes will be used to create computer models in collaboration with other groups. This will help us to predict the effects of disease and reduce the number of animals used in future projects.

Regarding the benefits on the treatment of atrial fibrillation, our mouse work will validate potential new targets previously identified in patients. In addition, our data will provide valuable information for future translational studies about the causal role of systemic inflammation on atrial fibrillation.

In the long run our investigations should provide answers as to whether epigenetic changes (Modifications of our DNA) driven by high levels of glucose may play a role in the development of cardiac dysfunction in diabetes. Again, these findings will open new avenues of treatment for diabetic patients. We also have the ability of teasing out the changes caused by diabetes in different areas of the atria. For instance, we will be able to track the effects of inflammation in the presence of diabetes, a process that cannot be quantified in patients using current imaging techniques.

In the short term, we hope that our work will provide original insights into the management and prevention of early metabolic triggers of diabetic cardiomyopathy and uncovers novel targets for the re- purposing of BH4-based therapeutics. If BH4 supplementation proves to be beneficial in our animal models we will be testing its efficacy in patients with heart failure. This process should not take long as BH4 is already a prescribed drug for patients with phenylketonuria.

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

We are collaborating with other groups with expertise in clinical trials, statistics and computer modelling inside and outside the establishment to have appropriate guidance and access to the latest methodology

Our results will be disseminated in national and international conferences and public engagement.

We are committed to publish negative data and unsuccessful approaches in order to prevent duplication of work and disseminate our research working with our funding institutions and other stakeholders.

**Species and numbers of animals expected to be used**

- Mice: 17500

## Predicted harms

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

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

Studying the role of changes in the NO-redox balance in myocardial disease requires us to carry out experiments in the intact organism. At present, it would be impossible to reliably model the behaviour of such a complex system using cell cultures or computer modelling. We will be using adult mice because this species is easy to manipulate genetically and has established models of disease similar to the human pathology.

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

The experiments we are proposing include phenotyping new genetically modified

mouse models as well as generating mouse models of human disease, notably diabetes and cardiac hypertrophy secondary to pressure overload (e.g., a situation that can be observed in patients with high arterial pressure or aortic valve stenosis) on which we could also test new therapeutic strategies. Treatment will be delivered orally, although in some cases we will be using intravenous injections or direct delivery of the drugs to achieve optimal effects in the heart. Alternatively, exercise (voluntary wheel running) will be used as an approach to reduce reactive oxygen species and increase NO availability.

Type 1 Diabetes will be induced by the administration of streptozotocin that is toxic to pancreatic beta cells, type 2 Diabetes by dietary modification or the use of transgenic models. Cardiac hypertrophy will be induced by aortic banding or by administration of angiotensin II or isoproterenol via an osmotic minipump implanted subcutaneously. The levels of glucose will be tested on these mice in order to confirm the presence of diabetes (similar to finger prick method in diabetic patients).

Characterisation of these models during a 6-10 week period would typically include non-invasive techniques such as echocardiography or MRI imaging, techniques that are similar to those used in the clinic. In order to characterise the impact of the disease and treatment on the atria we will apply external electrical stimulation to assess the probability of inducing arrhythmias on this organ. One or two of these examinations in total will be performed under general anaesthesia during the experimental protocol.

A final evaluation of cardiac function may be performed under terminal anaesthesia placing a catheter in the heart to directly measure pressure generation. At the end of the protocol mice will be killed humanely and further characterisation of the isolated heart or single cardiomyocytes performed to study the impact of disease and treatment at the organ/cellular/molecular level. In some of these mice we will be collecting blood in order to monitor the levels of the drug or quantify other metabolites.

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

Mice undergoing surgery will face adverse effects, e.g. pain, weight loss. We routinely apply pre- and post-operative care to the mice that undergo these procedures, ie, analgesia, heat support, access to water-softened chow, subcutaneous fluids and oxygen, application of liquid tears to the eyes and moistening the mouth after extubation. Isoflurane anaesthetic will be used for induction and full anaesthesia during surgery and cardiac imaging. Some mice will die under anaesthesia due to cardiac comprimise during horacotomy/TAC/myocardial injection. Monitoring will be increased during the recovery period and to prevent clinical signs of cardiac failure we will measure the function of the heart using ECG and cardiac imaging.

Diabetic mice will experience high blood glucose, excessive urination and thirst, therefore, they will be monitored and have free access to water and absorbent bedding will be added to the cage whenever necessary.

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

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

Most animals will not experience adverse effects as they will be used for breeding purposes or killed under terminal anaesthesia. Mice undergoing cardiac imaging will only experience mild severity during the recovery from anaesthesia. We expect moderate severity for those mice developing diabetes or mice that undergo surgery (approximately

15%). However, the <5% of animals that develop congestive heart failure might have a severe experience, since they may have difficulty breathing for several hours.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 22 July 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

Although we are actively engaged in the process of Replacement, we recognise that at present, it would be impossible to model the behaviour of such a complex system in silico or using cell lines. We are carrying out a number of complementary experiments in humans and surplus human tissue from patients undergoing cardiac surgery as well as developing a computational model of the healthy and diseased myocardium to guide our experiments and minimise the use of animals and in vivo experimentation.

In order to characterise the function of a specific gene on NO-redox balance we will initially make use of isolated cardiomyocytes and perfused heart preparations from mice, as these experiments are the least severe. To complete these first investigations and gain insights of the function of these genes in disease we will need to use models of human disease (i.e. diabetes, hypertrophy).

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

We are currently using non-animal alternatives to complete our research (e.g. computer modelling, genetic studies and human clinical trials).

**Why were they not suitable?**

Unfortunately, these approaches do not allow us to study the specific mechanisms and cannot replace animal experimentation.

For instance, the interactions of the heart with other organs (e.g. humoral signaling, immune and metabolic drivers) are lost when using in-vitro and ex-vivo experimental protocols and we cannot use computer modelling for complex process that develop over long periods of time (e.g. metabolic response or inflammation).

**A Retrospective assessment of Replacement will be due by 22 July 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 estimated number of animals has been calculated based on the usage of our previous licence. Most of the animals will be used in the breeding protocols. Our plan is to focus our efforts on the diabetes work but numbers may vary depending on the experimental results.

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

Numbers have been reduced in single cell based experiments (cell shortening,

calcium measurements and patch clamp, immunohistochemistry and molecular studies) due to the availability of three different cellular electrophysiology set ups as well as other equipment (e.g. confocal microscope/FRET system) on site, allowing us to have several scientists working on cardiomyocytes isolated from one heart.

To maximise the use of animals, we share our surplus tissue with other groups with non-cardiac research interests.

As part of the strategy to reduce the number of animals we are developing the optical mapping of the atria. This technique will allow us to assess cardiac electrical properties in perfused hearts instead of using live animals, thereby reducing the number of in vivo experiments.

The minimum numbers of animals required have been carefully reviewed by the funding agencies.

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

By carefully advance planning, we aim to closely match our experimental requirements with breeding output, and thereby avoid wastage. Our institution has a dedicated mouse colony expert who provides advice on efficient breeding. We freeze embryos or sperm from mouse lines that are not in routine use, since this avoids the need to breed mice simply to maintain a live colony. Wherever possible we will make use of pilot studies and share spare tissue with other groups that can use it and will

**A Retrospective assessment of Reduction will be** due **by 22 July 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.**

Surgical and imaging techniques are constantly refined and used by all the groups in our Department. Any step forward on the Refinement of these procedures that leads to minimise welfare cost to the animals is actively sought and shared in our regular animal welfare meetings.

We have modified the protocol to induce type 1 diabetes in mice. This allows us to generate a less severe model where we can study early changes in the myocardium. The severity and variability of previous protocols have been significantly reduced. Diabetic mice are subject to regular screening (blood glucose measurements and body weight monitoring). Careful attention is given to excess consumption of water or frequent wetting of bedding material. Absorbent bedding is added whenever necessary.

Refinements have also reached other techniques such as the Reduction in volume of blood required for glucose measurements or the delivery of drugs by using implants instead of multiple injections.

We routinely apply pre- and post-operative care to the mice that undergo procedures, ie, analgesia, heat support, access to water-softened chow, subcutaneous fluids and oxygen. Mice are allowed to recover in a heated chamber and checked after recovery. Recovery surgery is performed earlier in the day to allow sufficient monitoring within normal working hours.

We can use imaging and ECG monitoring to identify animals that are decompromising.

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

The human heart, in common with other mammals, has four chambers and generates normal pressures of 100-120 mmHg. Fish are not suitable for our studies as they heal and regenerate their hearts, while frogs have a 3-chamber heart and generate 30 mmHg. Mice are therefore the least sentient species that have a cardiovascular system and response to

disease that is sufficiently similar to humans. We need to use adults, because most heart disease affects adults. We will use terminal experiments where possible.

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

For diabetic mice, we will regularly monitor blood glucose levels and increase the frequency above 25 mmol/L and we will humanely kill animals if they lose >15% body weight. We expect the mice to be thirsty and urinate more, so we will check their water and change the cage bedding more often.

Pain and suffering during recovery of surgery will be mitigated by using aseptic technique and giving pain killers, fluids, softened food, and heat support during the recovery period. The majority of mice make a full recovery within days and will not experience further adverse effects.

We will increased monitoring and will use imaging or ECG to see subclinical changes indicative of future cardiac decompensation.

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

At the start of each experiment we will check 3Rs websites such as NC3Rs (https://www.nc3rs.org.uk/3rs-resources).

We will follow Home Office guidance on the "Code of practice for the housing and care of animals bred, supplied or used for scientific purposes". Guidance for aseptic surgery will be taken from "Guiding Principles for Preparing for and Undertaking Aseptic Surgery" (LASA 2017). At the experimental planning stage we will refer to the PREPARE guidelines checklist ("Planning Research and Experimental Procedures on Animals: Recommendations for Excellence") and to ensure our experiments are reported effectively we will adhere to the ARRIVE guidelines ("Animal Research: Reporting of In Vivo Experiments").

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

At the start of each experiment we will check 3Rs websites such as NC3Rs (https://www.nc3rs.org.uk/3rs-resources) and Norecopa (https://norecopa.no/3r-guide). We also receive regular newsletters from these and other organisations, our department holds animal welfare meetings three times a year where progress on the 3Rs is discussed, and there is a regular institution-wide newsletter on the 3Rs.

**A Retrospective assessment of Refinement will be due by 22 July 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?

# 5. Early detection and cardio protection in chemotherapy-induced cardiotoxicity

**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**

cardiotoxicity, chemotherapy, magnetic resonance imaging, cardio protection, metabolism

| Animal types | Life stages |
|---|---|
| Rats | adult, juvenile |
| 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 aim of this project is to 1) assess if the new imaging technique called hyperpolarized magnetic resonance imaging can be used for early detection of chemotherapy-induced cardiotoxicity and to 2) test existing drugs that boost energy generation in the heart as cardioprotective agents to prevent or reverse chemotherapy-induced cardiotoxicity while 3) preserving the anti-cancer effect of chemotherapy.

**A Retrospective assessment of these aims will be due by 06 July 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?**

It has been known for decades that some chemotherapeutic drugs such as doxorubicin have serious cardiotoxic side effects, which can lead to congestive heart failure and premature death in a proportion of cancer survivors (5-10%). Nevertheless, to this date there are no imaging markers available clinically to detect this toxicity early enough and there are no specific cardioprotective drugs to either prevent or treat chemotherapy-induced cardiotoxicity.

This is mainly due to the fact that the mechanism of chemotherapy-induced cardiotoxicity is still not completely understood. One predominant theory is that chemotherapy leads to oxidative stress in the heart, however, drugs that have antioxidant properties and prevent reactive oxygen species production have not shown clinical success.

Most mechanistic insight into chemotherapy-induced cardiotoxicity stems from mouse models where chemotherapy is injected in a single high dose into the intraperitoneal cavity, which does not represent the intravenous dosing regimen in patients. Intraperitoneal injection of chemotherapy can lead to inflammation and tissue necrosis at the point of injection, which may in itself lead to oxidative stress and toxic effects on the heart. More recent data from clinically more relevant models, including our own rat model of repeated intravenous dosing, point towards loss of mitochondria, the 'power-houses' of the cell, as well as changes in metabolism as a mechanism for cardiotoxicity. However, there remain open questions as to whether direct effects on metabolism affect mitochondrial health or whether damage and loss of mitochondria lead to inadequate metabolism.

The heart requires a constant supply of metabolic fuel to meet energetic demands and this process is thought to be perturbed in chemotherapy-induced cardiotoxicity, either due to loss of mitochondria or due to direct effects on metabolic pathways. Cardiomyocytes, the cells of the heart, contain at least 30% of mitochondria by volume, which is more than in any other cell type in the body, apart from skeletal muscle cells. This high mitochondrial content is vital to sustain the energy requirements that support cardiac contractility and thus adequate heart function.

Cardiac metabolism is perturbed in many cardiac diseases, such as after a heart attack, and changes in metabolism are thought to precede functional decline. Hyperpolarized magnetic resonance imaging can measure metabolism in real time in vivo, which tells us how efficiently metabolic process are working and whether this changes as a disease

progresses. This technique is thus promising as an early screening tool in chemotherapy-induced cardiotoxicity, as it may be able to detect changes in cardiac metabolism that precede changes in cardiac function. Clinically, heart failure is not apparent for many years and often first gives patients symptoms on exertion (such as climbing several flights of stairs). In order to tease out functional defects in our animals early, we may use compounds which increase heart rate, while the animal is being scanned under anaesthesia to assess cardiac function and metabolism. This may help identify early effects of the chemotherapy where treatment is more likely to be efficacious. Where we identify imaging biomarkers of ardiotoxicity we may use these as scientific endpoints in experiments using cardioprotective agents.

There are several existing drugs which promote mitochondrial number and function. For example, polyphenols such as resveratrol are thought to increase mitochondrial number and thereby improve energy generation and contractility. Some of these are in clinical trials for patients suffering from heart failure resulting from other causes and thus could easily be repurposed to treat cancer patients suffering from chemotherapy-induced cardiotoxicity. We therefore want to test some of these drugs in chemotherapy-treated rats to establish whether they can confer cardio protection. A potential cardioprotective drug would be required to not-interfere with the anti-cancer action of the chemotherapy, and so we will also test cardioprotective drugs in animals with tumours. Some of the tumours treated most frequently with cardiotoxic chemotherapy are aggressive breast cancers that occur in young women and leukaemia that incur in children. We therefore need to use juvenile and young adult animals for our experiments.

Chemotherapy also adversely effects other organs and tissues, such as the liver and skeletal muscle. The mechanism of toxicity may be the same as in the heart and by studying organs and tissues other than the heart with hyperpolarized MRI and with ex vivo tissue analysis we hope to be able to unravel the mechanism of toxicity and find a dug that not only protects the heart but also other organ function.

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

Our research will provide valuable insights into the mechanisms behind damage caused by cardiotoxic drugs and whether hyperpolarized MRI can serve as an imaging tool in different cardiotoxic drugs to detect changes before functional decline. Detection of a biomarker using hyperpolarized MRI could be directly translated into the clinic as a screening tool for cancer patients on cardiotoxic chemotherapy, as clinical hyperpolarizes for use in patients exist. There are currently four clinical polarizers in the UK but we anticipate that more will be installed as the technique develops. However, hyperpolarized MRI is still under development and therefore not widely available as yet. Therefore, if we discover blood biomarkers that correlate with hyperpolarized imaging findings, we propose to move these biomarkers into the clinic as well. We are investigating the use of existing drugs which we hypothesize to be protective against the cardiotoxicity of chemotherapy and which could be easily repurposed in patients. If we identify a drug that is widely available, it may be possible for this to be used routinely to prevent chemotherapy-induced cardiotoxicity without the need for pre-screening.

Our work will be published in peer-reviewed journals and presented at national and international conferences. The co-PIs have published over 200 papers which have been cited over 5000 times. Many of the research group are members of national and international societies, such as the British Society for Cardiovascular Research, the International Society for Magnetic Research in Medicine and the International Cardio-oncology Society, and regularly present their work at meetings of the societies. Previously our research has been published in the national press, and future work will be promoted in a similar way.

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

Hyperpolarized MRI is available for patients and the first clinical trials using this technique have been published. We can therefore rapidly translate our imaging biomarkers found in rodents into cancer patients on cardiotoxic therapy. This will allow early detection of cancer patients that may develop heart failure as a consequence of their cancer treatment.

An increased understanding of the mechanisms behind the damage caused by cardiotoxic drugs, and how they may be treated can lead to repurposing of existing drugs. These drugs would be able to go directly to phase 2 clinical trial, since any adverse effects would already be known, and these trials could be run in combination with a trial on early detection by hyperpolarized MRI. Nevertheless, it would take around five years for a drug to be validated through phase 2 and 3 clinical trials. Overall, this could lead to a reduction in the number of patients who develop heart failure as a consequence of cancer treatment (usually 5-10%), minimising morbidity and mortality and reducing long-term costs on healthcare systems.

A further and more immediate impact of our research outputs will be the sharing of new knowledge and techniques with the scientific research community.

This will stem from presentation at conferences worldwide and the high impact publications that will come out during the project duration.

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

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

**Species and numbers of animals expected to be used**

- Mice: 100
- Rats: 350

## Predicted harms

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

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

As metabolism and energy generation are linked to the function of the organs of the body, we need to study these effects in a living animal. In order to study how systemically administered chemotherapy can affect cardiac function and metabolism we need to use rodent models which have a four chambered heart and respond to chemotherapy and changes in metabolism in a similar way to that in humans. The bulk of our work is done in young adult animals. However, for development of deteriorating organ function and/or metabolism, animals may need to be kept for sufficient time to allow the changes to develop. In some cases, we may study juvenile animals. For example, the most aggressive forms of breast cancer are present in young women and leukaemia often affects children, who suffer most from long-term cardiotoxic side effects, so in our work to develop a drug against the adverse effects of chemotherapy on the heart we need to use juveniles as well as adult animals.

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

The typical animal will receive weekly intravenous injections of a single cardiotoxic chemotherapeutic agents for up to 8 weeks and will be anaesthetised for each of these injections. The animal will be imaged on several occasions and blood will be sampled from the saphenous vein.

Where possible, injections, blood sampling and imaging will take place during the same period of anaesthesia and no animal will be anaesthetised more than a total of 10 times during the protocol and no more than twice weekly on up to two occasions. Substances may be administered, for example as a cardioprotective agent, an imaging contrast agent or as a stimulus to increase cardiac work during imaging, which can help us to determine the efficiency of the heart. If substances cannot be administered during the time of anaesthesia, for example if daily administration of a cardioprotective agent is required, these substances will be preferentially administered via the drinking water or diet, wherever possible, or by subcutaneous injection if the oral route is not permissible for the substance. Where possible, intraperitoneal or intravenous injections will be avoided.

Some animals may have a subcutaneous tumour implanted ahead of chemotherapy treatment. Organs may be removed under terminal anaesthesia while the heart is still beating.

In some cases, an experiment may be performed under terminal anaesthesia, such as insertion of a catheter into a blood vessel to measure blood flow.

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

The control animals receiving neither a subcutaneous tumour and/or chemotherapy will have a mild- moderate experience due to repeat anaesthesia for imaging. Animals on the chemotherapy protocol do not gain weight to the same extent as wild type animals, and

towards the end of the chemotherapy protocol animals can become listless or show signs of distress. in our experience, these effects do not affect more than 10% of animals and usually do not appear until the last couple of weeks of the protocol. Animals will be monitored for signs of weight loss, changes in body condition, grooming or changes in behaviour and monitoring frequency will be increased if animals that show weight loss, decrease in body condition, lack of grooming or changes in behaviour. Where we identify early imaging markers of cardiotoxicity and cardioprotective agents we may be able to use these to refine our humane endpoints.

Animals receiving subcutaneous tumours may develop ulcerations of the skin if the tumour cells infiltrate the skin. The tumour may cause infection in rare cases. Animals with a tumour burden will be closely monitored and skin lesions or infections will be treated in consultation with the vets. Should the tumours reach the maximum size allowed by the protocol the animals will be killed.

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

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

Based on previous experience and taking into account that a cohort of animals may carry a tumour burden we expect 15% of rats to have a mild experience, 65% a moderate experience and 20% a severe experience. We will not use mice on protocol 2 and so will not have mice with both the tumour and the chemotherapy. Therefore, we expect 15% of mice to have a mild experience, 75% a moderate experience and 10% a severe experience.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 06 July 2026**

**The PPL holder will be required to disclose:**

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

## Replacement

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

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

We study the interplay between organ function and substrate metabolism, with a particular emphasis on the heart. As metabolism and energy generation are linked to the function of the organs of the body, we need to study these effects in a living animal. In order to study how systemically administered chemotherapy can affect cardiac function and metabolism

we need to use rodent models which have a four chambered heart and respond to chemotherapy and changes in metabolism in a similar way to that in humans. Furthermore, we need to study systemically administered drugs which could potentially be cardioprotective against chemotherapy-induced cardiotoxicity to assess whether these drugs are promising candidates to move into clinical research.

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

We use mouse HL1 cells, a cell line that behaves like mouse heart cells, to determine the mechanisms behind effects observed in our in vivo studies. We also use this cell line to explore new theories before taking experiments into animals. As it is not easy to get human heart cells, we use human stem cell- derived cardiomyocytes to help us determine whether effects seen in rats or mice are likely to also occur in humans. We will use the tumour cell lines that we will implant into animals to induce a tumour burden for in vitro experiments to confirm that they respond to the chemotherapy we intend to use in vivo.

**Why were they not suitable?**

HL1 cells provide a useful model but they only enable us to understand what is happening in heart cells in a monolayer, isolated from other cell types and from the mechanical stresses encountered by cells in the heart. We can generate engineered heart tissue, which is a 3D construct where the cells contract against flexible posts, and this is a better model of the environment cells encounter in vivo. However, the cells retain a very immature phenotype and do not behave like a true adult heart cell and these cell systems can only ever provide a part of the picture when one is studying the complex metabolic interplay between different organs in the body. The heart contains many other cell types in addition to the beating cardiomyocytes. For example, in response to the cardiotoxic chemotherapy, dying cells send out signals to the body's immune system and this attracts immune cells to the damaged region.

Some of these immune cells are beneficial and others are not, and it is much harder to mimic the effects of this immune reaction in a dish.

**A Retrospective assessment of Replacement will be due by 06 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

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

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

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

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

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

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

Where possible, we use tissue or organs from control animals not required for the designated study, or animals that do not fit the criteria for the designated study, for method development for ex vivo experiments.

Where possible, we will use historic data from control animals not receiving chemotherapy or a tumour burden to reduce the number of animals needed in future experiments.

**A Retrospective assessment of Reduction will be due by 06 July 2026**

The PPL holder will be required to disclose:

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

# Refinement

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

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

We use rats and mice because the hearts and other organs are structurally and metabolically close to the human heart. We predominantly use rats as their hearts are larger and heart rate slower, allowing for better quality imaging and providing more tissue for ex vivo analysis, which reduces animal numbers. However, we will also use a small number of mice for proof of concept studies to establish models of chemotherapy-induced cardiotoxicity, as we may want to use GAA models in future PPLs to generate clinically relevant mouse models of cancer with chemotherapy-induced cardiotoxicity. Before proposing these experiments, we need to ensure our imaging techniques are sensitive enough to the smaller and faster-beating mouse heart. To develop our model of chemotherapy-induced cardiotoxicity we administer chemotherapy intravenously under light gas anaesthesia, which minimises the risk of damage at the injection site and closely mimics patient treatment schemes.

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

In order to examine changes to metabolism and organ function in disease, we need to use a model that matches the human physiology, rather than a non-mammalian system. We use recovery anaesthesia for imaging because this means we can observe multiple data points from one animal rather than having to use 3 or 4 times as many animals to generate the same data. We also use recovery anaesthesia as we need to allow the effects of the chemotherapy and the tumour to develop over many weeks, and to measure the potential benefit of drug treatments. This could not be done under terminal anaesthesia.

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

In our experiments to investigate the effect of chemotherapy on heart function, we have changed the way we inject the drug to give a more consistent delivery and decrease the likelihood of inflammation and discomfort at the point of injection. Of the over 100 animals treated so far, less than 10% experience a reaction at the injection site, which is generally small and resolves quickly, whereas in the pilot study the high dose needed caused local inflammation and swelling in some of the animals.

We've introduced double-HEPA filtered air handling systems as much as possible into our workflows, both MRI and otherwise. As well as protecting operators, these will have

positive beneficial effects on rodents undergoing procedures: as well as increasing sterility, there is a lower likelihood of airborne scents being communicated between animals.

A dedicated rodent echocardiography machine has been purchased for cardiovascular research, which is more efficient for scanning animals than human systems previously used, and therefore can achieve better results with less time under anaesthesia for each animal.

In order to observe breathing motion of the animals in the MRI scanner, we have previously used an induction loop across the chest. This loop is effective, but the signal it provides can be obscured briefly during each MR pulse. We have recently installed a small warm and plastic balloon, to be placed underneath the animal, that enables more robust monitoring of breathing, thereby increasing scanning efficiency and decreasing the time the animal is under anaesthesia.We have close links with the oncology imaging group in the University and discuss techniques between the groups. We liaise with the veterinary staff and keep up-to-date on LASA guidance to minimise harms.

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

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

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

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

**A Retrospective assessment of Refinement will be due by 06 July 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?

# 6. Modelling Autoimmune Disease for Drug Discovery

**Project duration**

5 years 0 months

**Project purpose**

- Basic research
- 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**

Autoimmune, Therapy, Immunology, Multiple sclerosis, Inflammation

| Animal types | Life stages |
|---|---|
| Mice | 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.

- 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 objective is to provide a suite of advanced models that will allow pharmaceutical and biotechnology industries to test novel and existing drug candidates for use in autoimmune diseases such as multiple sclerosis (MS). In vivo models are combined with advanced in vitro assays that allow us to study the effects of the test drugs on the immune system.

**A Retrospective assessment of these aims will be due by 13 July 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

The direct outcomes of this project will be data that can inform the drug development programmes of our clients, allowing faster decisions on which candidate drugs are most likely to prove beneficial to progress to clinical trials and ultimately treat patients. Rates of autoimmune diseases are rising globally and cost the UK billions annually. Whilst there are some effective treatments for some of the most prevalent disorders, such as Type 1 diabetes, for others such as Multiple Sclerosis, with an estimated 130,000 cases in the UK, effective treatments are limited and some can have serious side effects. With rates of autoimmune disorders on the rise, there is still a need for better, safer and more specific drugs and therapies to treat and ultimately cure these life limiting diseases.

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

We provide a suite of advanced, models of human disease that will allow the pharmaceutical and biotechnology industries to test novel and existing drug candidates for use in autoimmune disorders such as multiple sclerosis. Information from studies performed are used to make decisions about the progression of drug candidates to clinical trials.

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

In the short term, these outputs will assist the pharmaceutical and biotechnology industries in making key decisions about their drug discovery pipelines and whether to progress a drug candidate towards clinical trial. In the long term, this benefits patients as it prevents exposure to ineffective therapeutic agents and also facilitates the development of novel drug candidates that may have substantial clinical benefits.

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

As work is performed solely on a commercial basis, for biotech and pharmaceutical clients, output will be subject to client confidentiality and therefore collaborative opportunities will be limited and at the discretion of the client, however, some of our previous clients have published our data in peer reviewed journals and presented at Scientific conferences. As dissemination of information may not be feasible, we will maximise the usefulness of the output through high quality experimental design and review of proposed experiments for scientific merit before proceeding with any work.

**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 shall use mice in these projects as the role of the immune system and the mechanisms of disease in these models closely mimic a lot of the aspects observed in the human disease. Therefore, use of these animals for drug development is directly relevant for the clinical treatment of humans.

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

The majority of the genetically-modified mice will be humanely killed to provide cells and tissues for use in the laboratory and will undergo no further experimentation. Where experimentation is conducted, procedures include simple vaccination injections to provoke an immune response. In some experiments we shall subsequently need to provoke inflammation in the brain to mimic MS. We shall also use models in which chemicals are administered which temporarily affect nerve function in the brain. Usually this produces a less severe form of impairment. All animals will be humanely killed at the end of the experiment.

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

Beyond our breeding program, the majority of the animals used will experience mild to moderate effects.

For the majority of studies, mild effects will only be endured at the point of immunisation.

Where inflammation of the brain is induced, this can lead to moderate or severe disease (paralysis, which normally resolves within two weeks). A small number (around 50) of the genetically-modified mice might spontaneously develop signs of this disease. When this is identified, those mice are humanely killed immediately.

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

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

For the breeding of genetically altered animals, it is expected that over 95 % will experience sub- threshold or mild effects.

Where immunisation or treatment is conducted it is expected that over 95 % will experience mild effects. The remaining 5 % may experience moderate effects but it is extremely unlikely that any would experience severe effects.

Where brain inflammation is induced, it is expected that over 80 % would experience at least moderate effects, with a portion of these (approx. 30%) potentially experiencing severe effects. The remaining 20% may experience mild effects.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 13 July 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?**

Importantly, many of our assays are performed in the laboratory, rather than in animals. These often use human immune cells and allows us to test how different immune cells respond to potential drugs.

However, once we have defined the most likely candidate drugs, we must understand whether these can in fact help to dampen the in-tact immune system and prevent inflammation of affected tissues.

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

We routinely perform in vitro assays using human cells in order to test drug candidates on cells of the immune system. The majority of our work is conducted using these in vitro assays, however, animal studies are often required downstream of this in order to test drug efficacy in a living system prior to clinical trials in humans.

**Why were they not suitable?**

In vitro assays are very useful at measuring the effect of drug candidates on particular cell populations and complex assays can be designed to model a lot of the processes that occur in the body. However, in vitro systems currently cannot model all of the aspects involved in cell activation, cell migration and organ inflammation that occur in vivo.

**A Retrospective assessment of Replacement will be due by 13 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to**

**design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

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

This estimate is the maximum number of animals predicted. As a CRO it is difficult to accurately calculate the exact number on animals that will be used as this largely relies upon client requirements and demand. Whilst we offer in vivo models as a service, these experiments are ordinarily only performed as a small part of a larger package of work that is primarily focused on in vitro models.

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

We have over 10 years ' experience with these models and as part of establishing these models we optimised the models so that we could use as few animals as possible but still obtain sufficient relevant information.

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

Our advanced immunology assays allow us to advise our clients on which of their test compounds are most likely to be effective in studies. This saves time and money and, most importantly, reduces the numbers of animals required for studies.

In addition, pilot/initial studies will be performed using the least invasive protocols and only using drug candidates that have been proven to be effective in in vitro experiments.

Where breeding is required, practices will be employed that prevent excessive over-breeding.

**A Retrospective assessment of Reduction will be due by 13 July 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.**

Our objective is to provide advanced, models of human disease that will allow the pharmaceutical and biotechnology industries to test novel and existing drug candidates for use in autoimmune diseases such as multiple sclerosis (MS). In vivo models are combined with advanced in vitro assays that allow us to study the effects of the test drugs on inflammation driven by the immune system within the brain.

The majority of the genetically-modified mice will be humanely killed to provide cells and tissues for use in the laboratory and will undergo no further experimentation. Beyond our breeding program, the majority of the animals used will experience mild to moderate effects. These include simple vaccination injections to provoke an immune response. In some experiments we shall subsequently need to provoke inflammation in the brain to mimic MS. This can lead to severe disease (paralysis, which normally resolves). A small number (around 50) of the genetically-modified mice might spontaneously develop signs of this disease. When this is identified, those mice are humanely killed immediately. All animals will be humanely killed at the end of the experiment.

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

The processes that lead to diseases such as MS are not fully understood, but include an unwanted immune response which in the case of MS occurs in the CNS, leading subsequently to nerve damage that cannot be repaired. These complex interactions cannot be fully replicated in less sentient species and mice have an extensively characterised immune system that closely resembles that of humans.

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

Where necessary, animals will receive appropriate anaesthesia and analgesia during and following the procedure and analgesia following surgery. In some experiments animals are at risk of infection. These animals are be kept in a barrier environment and receive appropriate antibiotics. All animals are monitored regularly and any that show signs of ill health receive prompt veterinary intervention. If significant ill health is evident, the animals are humanely culled. Any further refinements that can be implemented over the course of this projects will be put in place following consultation with the Named Animal Care and Welfare Officer (NACWO) and Named Veterinary Surgeon (NVS).

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

In addition to following the ARRIVE guidelines and the best practice procedures recommended by the NC3Rs (https://www.nc3rs.org.uk/), regular literature searched will be performed to ensure that the models used are the most refined for each purpose.

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

We will routinely access the resources on the NC3Rs website (https://www.nc3rs.org.uk/ ) and subscribe to the NC3Rs newsletter in order to keep up to date with current developments.

**A Retrospective assessment of Refinement will be due by 13 July 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?

# 7. Brain mechanisms for learning, planning and decision-making

**Project duration**

5 years 0 months

**Project purpose**

- Basic research

**Key words**

decision-making, learning, planning, prefrontal cortex, hippocampus and entorhinal cortex

| Animal types | Life stages |
|---|---|
| Rhesus macaques | juvenile, adult |

## Retrospective assessment

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

**Reason for Retrospective assessment**

This may include reasons from previous versions of this licence.

- Uses non-human primates
- 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 examine how neurons in the brain support learning, planning and decision-making.

**A Retrospective assessment of these aims will be due by 26 August 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Decision-making is one of the most important cognitive functions of humans and animals. We make hundreds of decisions every day, from the seemingly simple (e.g., what to eat for lunch) to the more complex (e.g., how to best allocate your time). Most of these decisions will be associated with their own costs and benefits (reward, time, probability, effort, etc) which collectively need evaluation and integration to determine each choice's overall value. Decision-making may also benefit from learning a model of the environment; that is, knowledge of the rules or structure of the environment (e.g., knowledge of the London transport system). Learning a model of the environment allows one to easily adjust behaviour when unexpected "transitions" occur (e.g., when your commuter bus/train line is down), but also such model knowledge can generalize and allow one to simulate or predict relevant or appropriate behaviour if placed in a novel environment (e.g., applying rules/concepts from the London Underground when using the Paris Underground). Thus, arguably the main function of the brain and its many cognitive functions (memory, learning, attention) is to support learning and decision-making processes so that we can plan accordingly and optimize behaviour.

Damage to the part of the brain called the prefrontal cortex (PFC) can be devastating, as humans and monkeys with damage to PFC are disorganized, impatient, make poor decisions and exhibit socially inappropriate behaviour. Moreover, dysfunction of PFC is associated with neuropsychiatric illnesses impairing choice, including depression, addiction, schizophrenia, obsessive-compulsive, anxiety and attention-deficit hyperactivity disorder. This implies PFC is an essential structure for guiding many of our everyday thoughts and behaviours. However, developing causal explanations of why brain dysfunction leads to abnormal behaviour must be understood at the level of the brain's main processing unit, the neuron. Neurophysiology (the recording of neural activity) is currently the only way to understand how single neurons encode information, and hence the only way to study the neural computations and mechanisms that support these cognitive processes. Such exploratory research is unethical in healthy humans since it necessarily involves inserting very fine wires into the brain to measure the activity of single neurons. Non-human primates (NHPs) such as the rhesus macaque have very similar brains to humans and exhibit advanced cognitive abilities. NHPs are therefore the best model for neurophysiology research about these cognitive processes.

This project seeks to understand how the brain supports learning, decision-making, planning and memory. We will record neurons in parts of the frontal and temporal lobes of NHPs as they perform learning and decision-making tasks, and use safe and reversible stimulation approaches to disrupt normal brain activity to identify which brain areas are essential for these cognitive processes. More specifically, we will explore whether neurons can 'replay' events forward or backward in time, which would facilitate learning and planning mechanisms. We will also examine whether neurons exhibit a particular pattern of activity called a 'grid cell' which would allow for information to be efficiently represented, hence facilitating learning, planning and decision-making. We also will develop new wireless approaches for recording neural activity in freely-moving NHPs, providing an important welfare refinement to neurophysiology research.

In the short-term, these studies will provide critical insight into the brain mechanisms that support learning and decision-making in the healthy brain. By understanding the functional organization of the normal brain, our research will provide a template for comparison with the abnormal brain, and hence in the longer-term aid the development of novel therapies for neurological disease.

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

Our research will generate an enormous amount of novel data to provide insight into the behavioural and neural mechanisms which support how information is learned, evaluated, and decided upon. This work should primarily be classed as "discovery" or "basic" research. However, our discovery research has potential for translational benefit in the long-term. For example, we will examine the neuronal mechanisms supporting reinforcement learning and planning, which will provide important insight into mechanisms of habit formation, such as in addiction. We will examine how information that is experienced when awake is consolidated during sleep to promote learning and form memories, and whether 'grid cells' provide a neural algorithm to efficiently link information together to form memories, concepts and rules; this research could provide important insight for studies of cognitive decline in ageing, as well as diseases of cognitive dysfunction (e.g., dementia). We will also use safe and reversible stimulation approaches to observe the effects of temporarily disrupting normal brain activity in specific regions, which provides a model for understanding how neurological disease affecting these brain regions may alter behaviour.

In the short-term (0-5 years), these studies will generate a mechanistic account of how neurons in the PFC and temporal lobe support some of our most important cognitive processes including learning, memory, planning and decision-making. In the longer-term (>5 years), this discovery research can provide a functional blueprint to aid the development of novel therapies for diseases that affect PFC and/or cognition.

**There are two main outputs of our research.**

Publications: Each of these experiments will lead to publications in peer-reviewed journals. We have a strong track-record of publishing in high-impact journals. Moreover, we typically publish at least two research articles (sometimes 3-4) from each study. Additionally, we are likely to publish review articles from this work. In total, we would expect at least 10 new publications from this research programme.

Technology Development and Transfer: We will develop new technology; specifically, we will build a system to wirelessly record neural data from non-human primates (NHPs) as they freely move in their home-room. The system will be freely open-source, providing a platform to share our research with other end-users internationally. This technology will allow the continuous mapping of the NHP brain during performance of diverse and naturalistic tasks, without the need for head restraint or separation from cagemates, hence an important welfare refinement. We believe this technology will radically transform how NHP neuroscience is performed.

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

While the proposed experiments address fundamental questions about the functional operations of the normal (healthy) brain, such a functional blueprint of PFC function can, in the longer-term, aid the design of therapies for patients with PFC dysfunction. The research aims to identify the fundamental brain mechanisms that support higher-order cognitive processes such as learning, decision-making, attention, planning and memory. The cognitive functions are known to depend on the PFC and its interactions with the temporal lobe. For example, humans or animals with damage to PFC are disorganized, impatient, indecisive, make poor decisions and exhibit socially inappropriate behaviour. Further, dysfunction of PFC is associated with neuropsychiatric illnesses impairing choice, including depression, addiction, schizophrenia, obsessive-compulsive, anxiety and attention-deficit hyperactivity disorder. For patients with intractable symptoms, current therapies include removing or disconnecting parts of the frontal cortex. Thus, in the long term, our research may lead to better understanding and treatments for these neurological diseases in which single or multiple cognitive operations are negatively affected. These cognitive disorders are a major health and societal burden, in addition to being often devastating to the individual.

Importantly, the benefits of this research are considerable. By simultaneously recording from many single neurons across the critical regions implicated in decision-making and higher-order cognitive processes, we not only aim to identify the unique functional computations served by these brain areas, but also examine how these regions functionally interact and communicate to optimize behaviour. In conjunction with our multi-site neuron recording approaches, we also use reversible inactivation/stimulation approaches which allows us to temporarily disrupt normal activity in a specific brain region to test its causal role in specific behaviours, and allow us to develop and test models of specific neurological disorders (e.g., psychosis). Such a mechanistic understanding of PFC operations would provide a blueprint for the development of rational therapies/targets for these psychiatric disorders. At present, there are no working alternatives to understanding the neural basis of the dysfunction of prefrontal cortex and its consequences for mental health.

At an academic level, the research will benefit a large academic community. One of our main aims is to test whether grid cells can encode non-spatial information; if so, this will be a major discovery as it may reveal the neural algorithm for how the brain connects and relates information together, hence supporting learning and the formation of memories. One of our other aims is to examine whether neurons can 'replay' events either forward (i.e., simulation) or backward in time; neural replay could provide a mechanism for supporting how we plan effectively, particularly when faced with novel situations. These experiments will thus have major appeal to academics working in cognitive and computational neuroscience, as well as people working in artificial intelligence research. The benefit will be in terms of increased knowledge about which cells in which brain areas are involved in learning, planning, memory and decision-making, what types of neural algorithms or computations are used, how distributed networks interact and communicate to support these cognitive processes, and how normal behaviour is altered when these computations or brain areas are disrupted. This will help other researchers (including

myself) to build better models of the functional organization of the normal brain, and hence provide a better blueprint for developing rationale therapies for the diseased brain.

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

We will maximise the output of the work in four ways:

- Research Publications: As described above, each of the experiments in this PPL will lead to publications in peer-reviewed journals. We typically publish at least two research articles (sometimes 3-
- 4) from each study. Additionally, we are likely to publish review articles from this work. In total, we would expect at least 10 new publications from this research programme.

Collaboration: We have assembled a strong collaborative Team. We collaborate with experts in human neuroimaging and perform analogous experiments in both humans and NHPs; e.g., we might use functional magnetic resonance imaging (fMRI) to identify regions where specific neural computations might exist. The experiments proposed here directly test whether grid cells encode non- spatial relational knowledge, and whether hippocampal replay supports planning mechanisms. These results will in turn inform future human experiments. We will collaborate with researchers who have developed non-invasive transcranial ultrasound stimulation (TUS). We will use TUS to directly test the causal role of PFC and temporal lobe structures and their underlying computations; this again will inform human and NHP experiments. We will collaborate with people that develop wearable neural data loggers, which can stably record single neuron activity of the same cells for many weeks during both free behaviour and during sleep. We will combine this datalogger with in-cage training systems, and share the integrated technology with the NHP community. We also collaborate with experts in data analysis, who will provide different analysis approaches to help us maximise the identification of functional signatures within our data. Finally, we also collaborate with experts in theoretical and computational neuroscience. Taken together, this Team of Collaborators will ensure our experiments are designed, and our data collected and analysed, to maximise the output of the research, including its translation to understanding the functional organization of the human brain.

Public Communication: In addition to our scientific publications, we routinely disseminate our research at international conferences, invited lectures and themed workshops. This also provides an opportunity to form new collaborations. To convey our research more broadly and to the general public, we engage with the University Press Office to distribute press releases for high-impact publications, and use social media to direct people towards key findings. We also give interviews and podcasts.

Data/knowledge sharing: This project will produce large amounts of data of interest to the wider neuroscience community including computational neuroscientists. Where possible, we will endeavour to make these data publicly available by posting them to public data archives. Data will be suitably annotated to facilitate interpretation. To ensure ease of replication in analysis, all processing of the data will be done using a scripting program that allows others to repeat the exact same procedures either on the same data or on

similar data. Our in-cage training system is also open-access. Other users of this system can upload their code so that resources can be shared within the larger community.

**Species and numbers of animals expected to be used**

- Rhesus macaques: 11

## 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**.

Humans and other primates with damage to the prefrontal cortex (PFC) are disorganized, impatient, make poor decisions and exhibit socially inappropriate behaviour. Further, dysfunction of PFC is associated with neuropsychiatric illnesses impairing choice, including depression, addiction, schizophrenia, obsessive-compulsive, anxiety and attention-deficit hyperactivity disorder.

Neurophysiology is currently the only way to understand how single neurons encode information. Such exploratory research is unethical in healthy humans since it necessarily involves invasive access to neural tissue. Our tasks are designed to explore the neural computations which support learning, decision-making, planning and inference, and are designed to directly parallel tasks that we also perform in humans. The cognitive requirements to solve our complex tasks are unlikely to be met in other species such as rodents. Thus our experiments are carried out in normal, healthy adult non- human primates (NHPs, Macaca mulatta), and will examine the activity of populations of neurons, to provide a mechanistic understanding of how PFC and interconnected areas support the high-level cognitive processes - including learning, planning and decision-making - that are often disrupted in these patients. Studies have noted remarkable functional and anatomical similarity of PFC structures between human and macaque monkey compared to human and rodent, which has emphasized the use of NHPs as an appropriate model for understanding human PFC function. These studies will have major implications in understanding decision-making in health and disease.

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

All NHPs are routinely pair-housed and are provided with an enriched environment and have home cages, exercise pens and forage areas. They interact regularly during the day with investigators and husbandry staff. The protocol involves a number of stages for preparing NHPs for recording neuronal data while performing behavioural tasks. This includes a number of separate and well-spaced surgeries under general anaesthesia, where biocompatible implants are fixed to the skull to allow for the study of the activity of individual neurons. These surgeries are carried out under full aseptic conditions and involve a full regime of pre- and post-operative analgesia. All general anaesthetic procedures are carried out by a Veterinary Surgeon (VS) and all surgical protocols are reviewed by the Named Veterinary Surgeon (NVS) prior to surgery.

An overview of the expected life experience of an NHP on this protocol is as follows. Each NHP will typically have two MRI scans to custom-design our cranial implants and confirm the location of recording electrodes. Behavioural testing sessions involve NHPs sitting in an enclosed testing chair, with access to a joystick or touchscreen monitor for interacting and responding to the experimental task. The NHP's head may be immobilized to allow infra-red tracking of eye movements and safe collection of neuronal data from both brain hemispheres while the NHP performs its trained task. In other cases, behavioural testing and wireless electrophysiological recordings are performed in the home-cage, without restraint. Neuronal data is collected from very fine, delicate electrodes that are fixed or advanced into the brain, which cause little or no harm to the brain, which cause little or no pain to the subject as the brain lacks pain receptors, and which are now routinely used in human neurosurgery without complications. Neuronal recordings are usually taken from multiple cortical and subcortical sites. We may safely and reversibly disrupt normal brain activity with neurostimulation and/or pharmacological approaches independent of, or in conjunction with, electrophysiological recordings; both are intended to cause only transient and minor cognitive effects that are safe and fully reversed before the NHP is returned back with their cagemate(s). NHPs may be on a scheduled or controlled fluid regimen at various stages of training to reward and maintain task motivation and dietary fitness, but the protocol is individually-titrated so that each NHP experiences the minimum amount of control (if any) necessary to ensure good task performance and that sufficient trials are obtained for data analysis. These experiments typically last 3 years.

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

None of our experiments are designed to induce pain or suffering. Although extremely rare, there are a number of possible adverse effects that could occur during/following surgery and anaesthesia (brain haemorrhage, seizure, paresis, breathing obstruction, failure of respiratory or cardiac systems) or from insertion of recording electrodes into the brain (brain haemorrhage, seizure, paresis). Of these rare events, seizures are the most common, though these typically subside immediately with medical treatment. Our research team places special emphasis on pre- and post-operative monitoring and care of the highest standard, including use of analgesics in consultation with the Named Veterinary Surgeon (NVS). Long-term cranial implants can be associated with infections, though the implants are bio- compatible and regularly inspected and cleaned, such that any infections are quickly detected and appropriate antibiotics are used. In the rare event that an implant fails or if the animal's health is compromised by the implant (e.g., unresolvable infection), surgery may be performed to repair/remove the implant(s); the implants may then be re-implanted months later if the animal's health returns to normal. To perform the experimental procedures the animal's head is restrained which can induce anxiety and distress; to mitigate this, the training and restraint duration is slowly increased over many days and paired with positive reinforcement (i.e., juice reward) to reduce anxiety and distress. Neuronal data is collected from very fine, delicate electrodes that are fixed or advanced into the brain, which cause little or no harm to the brain, and cause little or no pain to the subject as the brain lacks pain receptors. NHPs may be on a scheduled or controlled fluid regimen at various stages of training to reward and maintain task

motivation and dietary fitness, but the protocol is individually-titrated so that each NHP experiences the minimum amount of control (if any) necessary to ensure good task performance. Food and fluid intake will be constantly monitored and recorded to verify a stable weight and health of the animal; all animals are expected to maintain good health and weight during the protocol.

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

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

Great effort and care is taken to minimize the suffering for each NHP, including use of analgesics as necessary and as advised by the Named Veterinary Surgeon (NVS). We continually refine our methods and surgical approaches to improve overall outcomes. We use biocompatible implants which improve implant stability and overall implant health. This application employs several refinements in behavioural training (e.g., home-cage training without restraint) and electrophysiology approaches (e.g., higher yield electrodes, wireless electrode approaches) which are expected to yield better data, in fewer recording sessions, and with less overall restraint. Given these refined approaches, we expect that the life experience for most of the NHPs in this PPL will fall within a ‘Moderate’ severity limit.

Nevertheless, the invasive nature of the procedures is associated with some small risk for adverse effects (e.g., intracranial infection, cerebral haemorrhage, seizures) and thus in the worst-case scenario, there is a 10-20% chance the life experience of an NHP on this PPL could reach the ‘Severe’ limit.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 26 August 2026**

**The PPL holder will be required to disclose:**

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

## Replacement

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

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

This project seeks to understand how the brain supports higher-order cognitive functions, including learning, decision-making, planning and memory. These cognitive functions are commonly associated with interactions between the prefrontal cortex (PFC) and temporal

lobe structures, and are impaired in many neurological disorders. Developing causal mechanisms of why brain dysfunction leads to abnormal behaviour must be understood in the context of the functional circuitry at the single-neuron level. Neurophysiology (the recording of neural activity) is currently the only way to understand how single neurons encode information, and hence the only way to study the underlying neural computations that support these cognitive processes. Such exploratory research is unethical in healthy humans since it necessarily involves invasive access to neural tissue. The PFC of the non-human primate (NHP) brain has considerable anatomical and functional overlap with human PFC, but is virtually non-existent in other mammals, necessitating the use of NHPs in this project. Further, the cognitive and sensory-motor requirements of these tasks require a highly developed PFC, and such requirements could not be met in other species such as rodents.

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

Neurophysiology is currently the only way to understand how single neurons encode information. Such exploratory research is unethical in healthy humans since it necessarily involves invasive access to neural tissue. Non-invasive non-animal alternatives such as human neuroimaging and computational modelling are used to refine our hypotheses and design better experiments, but they cannot replace the information gained by recording from individual neurons.

**Why were they not suitable?**

Experiments in healthy humans rely on non-invasive measures (fMRI, PET, EEG, MEG) to assess brain function, connectivity, and cognitive processes. These approaches are valuable to understand human brain function to a limited extent. The spatial resolution of EEG/MEG are not adequate to capture the function of single neurons. Because fMRI measures the blood flow associated with the metabolic demands of neural activity, it has poor temporal resolution, on the order of several seconds after the neural event. Whilst these imaging approaches have the advantage of measuring activity over much (or all) of the brain, these methods can never test hypotheses or predictions about the firing patterns of individual neurons, or reveal the nature of that processing. To directly examine neuronal processing and communication, single neuron recordings are therefore required, so far without alternatives. Such exploratory research is unethical in healthy humans since it necessarily involves invasive access to neural tissue. Finally, theoretical models rely on experimental data to become generated and refined. Therefore, there is no alternative to experimental work involving animals. By combining the experimental approaches only available in animal studies with fMRI (performed in humans) and computational modelling, we attempt to bridge the methods gap and aim for insights from animal studies that can be informative for human studies and translational approaches.

**A Retrospective assessment of Replacement will be due by 26 August 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?**

For NHP electrophysiology studies, the generally agreed standard in the field is that results from 2-3 NHPs must be reported in a publication to ensure reliability of the results. The standard, driven mainly by ethical, but also scientific considerations (e.g., no hypotheses about inter-animal differences) is to use samples of within NHP comparison for power, and use more than one NHP for reassurance against idiosyncratic behaviour. This approach reflects the effort to keep the number of animals to a minimum. It is scientifically valid and justified, as experimental manipulations (e.g., reversible interference approaches) need to be compared to conditions in the same NHP with and without manipulation. Reliability is achieved by repeating the manipulation and measuring its effect many times, and by applying single-subject statistics (e.g., permutation testing) ensuring that experimental observations are systematic and do not arise only by chance. This approach works particularly well in electrophysiology studies, as neuronal response effects are typically seen in a single trial reflecting large effect sizes due to increased signal- to-noise ratios of having the recording device (i.e., electrode) located directly at the neuronal origin. Therefore, the data obtained from each NHP are usually highly significant, and consistent data from a second NHP supports the reliability and validity of the findings.

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

We have reduced the number of animals used to the fewest possible (n=2). For NHP electrophysiology studies, the generally agreed standard in the field, implemented by the major scientific publishers, is that results from 2-3 NHPs must be reported in a publication to ensure reliability of the results. This standard, driven mainly by ethical, but also scientific considerations (e.g., no hypotheses about inter- animal differences) is to use samples of within NHP comparison for power, and use more than one NHP for reassurance against idiosyncratic behaviour. This approach reflects the effort to keep the number of animals to a minimum whilst maintaining scientific validity.

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

To ensure reproducibility of our methods and findings, following also ARRIVE guidelines, we provide complete descriptions of our methods and data in our publications. Where possible, we will endeavour to make these data publicly available by posting them to public data archives. Data will be suitably annotated to facilitate interpretation. To ensure ease of replication in analysis, a scripting program will be used that allows others to repeat the exact same procedures either on the same data or on similar data.

Whilst it is not currently possible to develop non-animal alternatives to electrophysiology research, we perform analogous experiments in humans (both behavioural and neuroimaging studies), and invest considerable energy in computational modelling of the potential circuits and neural computations supporting the cognitive operations examined in our tasks. These studies in humans shape and inform our NHP studies; they allow us to make specific predictions about the types of neural computations expressed during performance of our tasks. The studies also inform where these neural signals might be located in the brain (hence refining where we target our single neuron recordings), and the potential consequence of disrupting these neurons/circuits with reversible interference approaches. Thus, these studies in humans refine our NHP experiments, thereby providing both a reduction of NHPs used and a refinement to the experimental approach.

**A Retrospective assessment of Reduction will be due by 26 August 2026**

**The PPL holder will be required to disclose:**

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

## Refinement

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

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

This project seeks to understand how the brain supports higher-order cognitive functions, including learning, decision-making, planning and memory. These cognitive functions are commonly associated with interactions between the prefrontal cortex (PFC) and temporal lobe structures, and are impaired in many neurological disorders. Developing causal mechanisms of why brain dysfunction leads to abnormal behaviour must be understood in the context of the functional circuitry at the single-neuron level. Neurophysiology (the recording of activity of individual neurons) is currently the only way to understand how single neurons encode information, and hence the only way to study the underlying neural computations that support these cognitive processes. Such exploratory research is unethical in healthy humans since it necessarily involves invasive access to neural tissue. Non-invasive non- animal alternatives such as human neuroimaging and computational modelling are used to refine our hypotheses and design better experiments, but they cannot replace the information gained by recording from individual neurons.

Our choice of species is macaque monkeys (Macaca mulatta) because they are the best available model for understanding the mechanisms of advanced cognitive function that characterise human cognition. First, our experiments are designed to examine the neural

basis of some of the most complex cognitive processes present in primates including humans (e.g., learning, decision-making, planning, inference). To solve these tasks, subjects must learn about the meaning of large sets of stimuli, learn common rules across many stimuli, and make inferences about what choices to make in novel situations; it is extremely unlikely that rodents could reliably distinguish between, and memorize, hundreds of visual objects required to perform these tasks correctly. Research over the past decades from many labs throughout the world has established that macaque monkeys can be well trained on various cognitive tasks involving controlled eye movements. This is not possible to the same extent in non-primate species. Second, these cognitive processes are known to rely on the prefrontal cortex (PFC) and interconnected brain structures. There is remarkable functional and anatomical similarity between the human and macaque monkey PFC, whereas PFC is largely absent in rodents. For our results to be applicable to human patients and understanding human PFC function, it is necessary that the work is carried out in macaques.

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

The aim of our research is to understand how the brain supports complex cognitive processes such as learning, planning, decision-making and inference. To study these cognitive processes, we use tasks that require learning and discriminating many stimuli, learning and generalising common features/rules from those stimuli, and making inferences in novel contexts, often with behaviours indicated via eye movements. Rodents lack the visual acuity to discriminate so many stimuli. Moreover, research over the past decades from many labs throughout the world has established that macaque monkeys can be well trained on various cognitive tasks such as these that require controlled eye movements, but this is not possible to the same extent in marmoset and rodents. Second, these cognitive processes are known to rely on the prefrontal cortex (PFC) and interconnected brain structures. There is remarkable functional and anatomical similarity between the human and macaque monkey PFC, whereas PFC is largely absent in rodents. For our results to be applicable to human patients and understanding human PFC function, it is necessary that the work is carried out in old world primates, such as macaques.

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

Our institution and research teams are fully committed and equipped for highest quality experiments in NHPs. Our research team places special emphasis on pre- and post-operative care of the highest possible standard, including use of analgesics in consultation with the Named Veterinary Surgeon (NVS). Food and fluid intake will be constantly monitored and recorded to verify a stable weight and health of the animal. Animals are also monitored daily during the week, including continuous CCTV, so any changes in their body condition, behaviour or overall health can be rapidly detected and any necessary changes to their schedules or veterinary treatments made promptly. We have developed effective methods for pair/group housing and social well-being of NHPs. We create rich environments for our NHPs, including exercise pens with natural daylight, toys, puzzles, foraging material and other cage furniture, as well as music and videos in their home

room. Given our research aims to identify “value signals” in the brain, it is imperative that NHPs find our experiments rewarding and hence valuable. As such, we take great effort to make the sure the NHPs are comfortable in the testing chairs (when needed), and that the tasks are interesting and motivating to the animal, by providing a variety (and choice) of preferred juice types, changing the stimuli or providing new task problems to solve. We also provide an assortment of healthy treats as part of their diet, and typically reward animals with fresh fruit following good performance during training sessions. As a result, NHPs voluntarily participate and work hard on our tasks, such that we are consistently able to collect the behavioural and neuronal data necessary to achieve our scientific aims.

We also continually refine our methods to promote best practice. We survey the literature closely, and refine implant designs to promote biocompatibility and long-term health and stability. We have also interacted with various working groups to develop better implants and surgical approaches. We discuss our surgical approaches with human neurosurgeons and human craniofacial surgeons, and they have attended our surgeries and advised on best practice. Our electrophysiological methods are also continually refined as new technologies emerge, so that we can obtain better neural data with fewer electrodes, and fewer recording sessions; this reduces the duration an NHP remains on protocol, which reduces the risk of any adverse effects. We are also developing wireless electrophysiology technologies so that experiments can be performed within the home-room, without restraint or separation from cagemates; these technologies will ultimately require fewer surgeries, and allow for more diverse and naturalistic behaviours to be explored, hence offering many refinements.

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

We continually strive to refine our approaches. This can come in at least two forms. First, we follow published best practice. This might be in relation to published reports on i) surgical approaches and/or implant design/materials (e.g., materials to promote biocompatibility of implants), ii) new neural recording technologies (e.g., the development of new electrodes; development of better software to isolate neurons and improve neuron yield) or in the form of training and housing protocols (e.g., use of food/fluid schedules; use of positive reinforcement training). Second, we also continually interact with stakeholders more informally where new data is shared, but perhaps not yet published. This might be in attending local 3Rs/NC3Rs events, attendance at welfare-related meetings and workshops, or collaborations and interactions with other NHP labs to learn about their best practice approaches. This allows us to stay up-to-date on current best practices, and learn how to safely incorporate these refinements into our own research approaches.

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

Our methods conform to those used in many primate neurophysiology laboratories for several decades around the world, and follow recommendations established by NC3Rs reports. Members of our lab routinely attend local 3R events, and attend or participate in

European meetings regarding animal welfare, and to discuss refinements and best practice procedures. We also regularly visit and interact with other NHP labs in the UK to discuss new approaches and refinements of current approaches. We interact with, and attend NC3Rs events, and remain updated with their published reports. We also regularly review the scientific literature and incorporate suggested refinements where safely possible (e.g., with implant design, wound protection, food/fluid control schedules, effective training methods, etc.).

**A Retrospective assessment of Refinement will be due by 26 August 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?

# 8. Promoting proliferation in non-regenerative tissues

**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**

Regeneration, Myocardial infarction, Heart failure, Cardiomyocyte, Therapy

| 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?**

Our overall aim is to determine if the heart and other non-regenerative tissues can be stimulated to re- enter cell cycle and promote regeneration post damage.

**A Retrospective assessment of these aims will be due by 12 August 2026**

**The PPL holder will be required to disclose:**

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

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these**

**could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

Significant injury to non-regenerative tissues, such as the heart, can have life-threatening or disabling consequences. Considerable research efforts are aimed at regenerating these tissues, with limited progress to date from a clinical perspective. Therefore, heart disease remains the leading cause of premature death in the UK. The proposed project aims to determine whether it is possible to stimulate regeneration of mouse hearts, following injury by an induced heart attack (myocardial infarction) that damages the heart muscle. The work will;

Provide new insights into the mechanisms required for regeneration.

Develop a method to regenerate injured heart muscle after a heart attack.

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

This project will yield a significant advance in the science underlying the failure of an organ, like the heart, to regenerate. The project will inform the development, and test the feasibility of novel therapeutic strategies to treat heart failure. We will also determine whether a similar regenerative strategy is applicable to the cells of the brain and the spinal cord, where there is also a major clinical deficit in regenerative therapies.

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

Heart disease is the leading cause of premature death in the UK. Heart failure is a common end-point of heart disease. There is currently around 1 million (23 million worldwide) heart failure patients in the UK, a disease burden that has a significant socio-economic impact. Mortality rates are around 50% of all cases, and heart failure is estimated to cost over £23 billion a year worldwide. There is currently no effective treatment other than cardiac transplant, and other treatments only slow disease progression.

In the short term, we are designing and developing new regenerative therapies, which can result in a localised, transient burst of protein activity. If successful, the next stages will be further preclinical studies of regeneration after an experimental heart attack in other animal models before early-stage clinical trials.

Over the long term, a successful therapeutic strategy for the treatment of heart failure would be transformative for patients, enhancing both lifespan and quality of life. Similarly, the development of a clinically effective neuronal regenerative strategy would have far-reaching applications in the NHS and globally. Apart from this societal impact, the economic and commercial value of effective therapeutic agents for either condition would be substantial.

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

We will make our models and research tools available to other researchers and will publish our finding in high impact journals. The information will be of interest to us, the biotechnology sector and to other groups researching similar ways regenerate damaged tissues. We expect to recruit talented postdocs and doctoral students and develop their scientific potential through the studies outlined in this proposal. Data generated will be deposited in publicly accessible databases for use by the research community. The data will be disseminated by presentations at international scientific meetings and seminars. The optimal route for translational development of the work will be considered and will be protected by patents as appropriate. A successful outcome would be a major step forward in regenerative medicine and in the long term, the work may lead directly to new lifesaving therapies for millions of patients.

**Species and numbers of animals expected to be used**

- Mice: 7900

## 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 genome (DNA) of the mouse has been well characterised and can be easily manipulated. We have generated switchable DNA modification to allow rapid activation (and deactivation) of proteins/genes within specific tissues. These models have enabled us to understand why some non-regenerative cells do not repair organs after damage. These mouse models represent the most suitable system in which to perform these studies.
- One non-regenerative tissue is the heart. Following an injury, the adult mammalian heart can lose up to a billion cardiomyocytes (the muscle cells of the heart) within the first few hours. It is unable to regenerate by replenishing lost cardiomyocytes. We will attempt to activate regeneration in the adult mammalian heart. Therefore, in the most part, adult mice will be used in this project.
- The induction of heart attack (myocardial infarction) in the mouse is the most commonly used method for studying ischemic heart disease (where the supply of blood is restricted) in mammals. It represents the current gold standard for investigation of mechanisms for heart repair and regeneration. Cell models in the laboratory do not adequately recapitulate the interactions in which cardiac regeneration occurs.
- The mouse represents the lowest level of sentience available to study mammalian heart development and disease.

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

After the generation of genetically modified animals by breeding or acquisition of mice from other sources, mice will be assigned to a protocol.

Typically, juvenile genetically altered mice will be administered with a virus or agent. This will lead to the expression of a gene specifically in an organ, such as the heart. In some rare cases, these agents will be injected directly into the heart tissue, which may require surgical access. A non- toxic protein activating agent will be administered by injection. This second agent will transiently activate a switchable protein and should induce the cells to reproduce (proliferate). During proliferation, we will label newly made cells by injecting or feeding a specific labelling agent. Organ function will be assessed by non-invasive monitoring (e.g. ultrasound scan) before and after protein activation, which may require repeated anaesthesia. Alternately, organ function will be assessed by a surgically inserted device that can monitor heart function over an extended time. Food may be withdrawn for short periods. Blood samples may be taken to monitor mice. Mice will be killed humanely at specific time points, usually in days or weeks.

Typically, juvenile genetically altered mice or wild-type mice will be administered with a virus or agent. This will lead to the expression of a gene specifically in the heart. At adulthood, mice will undergo experimental heart attack by injury of a coronary artery, which requires surgery to access the heart. In some cases, a gene inducing agent will be injected directly into the heart tissue. Food may be withdrawn for short periods. Blood sampling may be used to assess biomarkers of MI. Some animals will not be recovered from myocardial infarction surgery. After recovery from surgery, a non-toxic protein activating agent will be administered by injection. This second agent will transiently activate a switchable protein and should induce proliferation. During proliferation, we will label newly made cells by injecting or feeding a specific labelling agent. Heart function will be assessed over a time course, usually 28 to 56 days by non-invasive monitoring which will require repeated anaesthesia. Alternatively, heart function will be assessed by a surgically inserted device that can monitor over an extended time. Mice will be killed humanely at specific time points.

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

The vast majority of animals on this licence will suffer no or only minimal side effects (such as the effects of a small ear notch being taken for DNA analysis or the effects from a needle injections).

Our experimental models are designed to induce proliferation in normally non-regenerative tissues such as the heart. We have developed switchable tissue-specific technologies so that proliferation is localised and transient so will cause the least suffering for the shortest period. Nonetheless, the nature of the experiments will cause inevitable adverse effects, such as transient weight loss, a hunched posture and inactivity.

Our scientific goals are to test and develop new treatments for heart attacks and therefore rely on being able to model the regeneration of the adult human heart in the mouse. A heart attack is a serious condition and frequently leads to death in patients, so these animal protocols are severe in category, which relates to the levels of pain, suffering, distress and lasting harm caused. The induction of myocardial infarction in the mouse is the most commonly used method for studying ischemic heart disease in mammals and

represents the current gold standard for investigation of mechanisms for heart repair and regeneration. Approximately half of the mouse deaths occur during the initial surgery to cause a heart attack when the animal is anaesthetised and so experiences no discomfort. Most of the rest occur as sudden death at varying periods postoperatively. Heart attack in humans can result in pressure or tightness in chest, shortness of breath, sweating, nausea, vomiting, anxiety, cough, dizziness, fast heart rate and pain so suffering and pain in mice cannot be ruled out. In rodents who experience sudden death, loss of consciousness is likely to be immediate while death will occur within minutes so that suffering is not prolonged. Extensive monitoring will be used to detect signs of imminent cardiac arrest, in which case animals will be killed humanely. The other potential complication is heart failure, which may be detected by signs of cyanosis (bluish cast to the skin), oedema of the extremities (swelling in the feet and ankles) and laboured breathing, in which case animals will be killed humanely.

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

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

The majority of animals (up to 90 %) on this licence will experience either no, or only mild, transitory and/or minor pain or suffering. Any animals that undergo surgery (around 10 %) are likely to experience transient post-operative pain and discomfort, but no significant disturbance of an animal's normal state.

Some animals (less than 2 %) will experience short-term moderate pain, suffering or discomfort from extensive activation in proliferation in some organs. If the animals are suffering, they will be given suitable pain relief treatment, and if this does not alleviate the suffering promptly, they will be killed humanely. Some procedures may result in the death of a proportion of animals (less than 1 %), death usually occurs suddenly with only transient suffering. These models are used to find targets or test treatments that could regenerate the heart after a heart attack. These are complex pathologies that cannot be modelled in non-animal models. These experiments would only be undertaken after extensive pilot work to demonstrate the mechanisms underlying the pathology. All other animals will be killed humanely at the end of the studies. The enormous burden and severity of heart attacks in patients warrant the use of this category of animal protocols in order to find new treatments.

**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 12 August 2026**

**The PPL holder will be required to disclose:**

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

## Replacement

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

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

The heart represents a highly complex organ where coordinated interactions between cells, connective tissue, growth factors and blood flow cannot be accurately reproduced in culture. Thus, animal experiments are essential to understand regulatory mechanisms and to design and test potential new therapies. Given the complexity of the response of a damaged heart, no suitable alternatives exist that can entirely replace animal studies. Lower vertebrates such as zebrafish or newts have the capacity for cardiac regeneration. They are therefore useful to define factors that lead to regeneration, but they cannot be used to model the lack of regeneration seen in mammals. The mouse represents the lowest level of sentience available to study mammalian heart development and disease.

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

The muscle cells of the heart, the cardiomyocytes, can be generated from mouse or human embryonic stem cells which can be differentiated into specialised cells.

These newly differentiated cardiomyocytes model immature (neonatal) cardiomyocytes. Adult rodent cardiomyocytes can be purified and survive in culture for a few days. Where possible animal studies will be complemented by cell culture experiments.

**Why were they not suitable?**

Embryonic stem cell-derived cardiomyocytes display a very immature phenotype in which regeneration is apparent. They cannot be used to model the lack of regeneration seen in adult cardiomyocytes.

Purified adult cardiomyocytes do not survive long term in culture. Both cell culture models do not adequately recapitulate the cellular context and interactions in which cardiac regeneration occurs. Furthermore, cells in culture are subject to an environment very different from that in a live animal. Cells in culture experience a variable and abnormal oxygen tension, are usually cultured in a vast excess of glucose and ill-defined growth factors, survival factors and secreted substances derived from cows.

Although three-dimensional culture methods (organoid cultures) offer some advantages, they are severely limited by the rather crude and unrepresentative cell scaffolds that are used and cannot reconstitute the complex interaction of multiple cell types. It is also not possible to reconstitute a functional immune system that has been shown to respond to regeneration. Therefore, cell culture systems cannot be used to model the complex interaction of multiple cell types that responds during regeneration and are not suitable surrogates as models of heart regeneration.

**A Retrospective assessment of Replacement will be due by 12 August 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?**

Controls will include sham surgical animals (animals that have undergone surgery but no heart attack induced), genetic controls and vehicle treatment controls. Sham controls will estimate heart function after no damage.

Estimation of numbers will be informed by an analysis of published work and preliminary experiments and with assistance from a statistician. We will use power calculations.

Variability between experimental groups will be limited by using closely related mouse strains raised in a controlled environment, free from specific diseases, fed a uniform diet and matched for age and body weight. Imaging and analysis will be performed by blinded and experienced users.

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

- The experiments have been designed with a number of points in mind to minimise animal use and animal suffering.
- We will use well-validated systems and mouse models in which we have extensive experience.
- Previous studies by our collaborators on mouse models of heart attack and regeneration have already identified the most appropriate techniques required for consistent results and the time points at which the models provide the most information.
- The use of non-invasive imaging (ultrasound/ magnetic resonance imaging) of heart function as well as telemetry (implants for monitoring mouse health) will gather more data from each animal and gain extra control over variability – reducing the number of animals required used per study.
- The experimental group sizes will be based on pre-existing data. Importantly, each animal can act as its own control when analysing non-invasive imaging, ensuring that the experiments do not need to be repeated unnecessarily.

- Mice will be randomly allocated to experimental groups, maintaining comparable segregation of age, size and gender. All animals will be maintained in the same environment. The same person will administer experimental agents (or control substances) and retain a key to identify recipient mice. This key will only be available after analysis has been carried out.
- Many of our experiments require animals with complex genotypes. We will carefully plan breeding strategies to minimise the number of animals of incorrect genotype.
- At every stage, we carefully examine and question our need for animals and have consequently adopted whatever strategies possible to reduce their use.
- We will use published guidelines to aid in the planning, design, analysis and reporting of all studies (https://norecopa.no/PREPARE and www.arriveguidelines.org).

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

Since most of our experimental animals have complex genotypes, we have carefully planned breeding strategies to maximize the number of suitable experimental animals and control littermates.

Wherever possible, individual mouse hearts will be used for multiple assays (e.g. one heart is divided into two to generate material for multiple purposes). Where possible (e.g. where tissues can be used as controls for other experiments) mouse tissues will be shared amongst the research group. This will maximize the amount of information that can be acquired from the minimum number of animals.

Pilot studies will be conducted to determine the feasibility and efficacy. Advice will be sought from an internal departmental expert statistician.

**A Retrospective assessment of Reduction will be due by 12 August 2026**

**The PPL holder will be required to disclose:**

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

## Refinement

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

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

The mouse is the ideal animal since it is readily amenable to genetic modification and models of injury.

The genome of the mouse has been well characterised and can be manipulated by gene targeting. We have generated switchable genetic modification to allow rapid activation (and deactivation) of genes within discrete tissues; therefore these mouse models represent the most suitable system in which to perform these studies.

The induction of heart attack (myocardial infarction) in the mouse is the most commonly used method for studying ischemic heart disease in mammals and represents the current gold standard for investigation of mechanisms for heart repair and regeneration. The mouse represents the lowest level of sentience available to study mammalian heart development and disease.

Similar studies investigating the role of individual genes in heart development and regeneration cannot be performed in humans or other non-animal models.

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

Lower vertebrates such as zebrafish or newts have the capacity for cardiac regeneration so cannot be used to model the lack of regeneration seen in adult human hearts.

Our scientific goals rely on being able to model the regeneration of the adult human heart in the mouse. Mouse physiology is sufficiently similar to that of humans to generate passable representations of the human disease. This is facilitated by detailed knowledge and comparison of mouse physiology and genetics.

Cardiac regeneration diminishes during mammalian development, and therefore, regeneration needs to be assessed in adult mice. It is not possible to use more immature stages or terminally anaesthetised animals.

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

- We will continue to use rapidly switchable models that allow predictable short-term outcomes to limit welfare costs to the animals.
- We will ensure that the animals are regularly monitored by experienced workers so as not to exceed pre-determined endpoints that might increase the amount of distress caused.
- Staff will be trained and become experienced in surgery. Pilot studies will be performed in undamaged hearts to establish the best treatment schedules, reducing the total number of mice required. We will carry out surgeries early in the day, allowing intensive monitoring in the afternoon and evening. Mice will be monitored twice daily over the next three days, and every day after that to ensure we detect any suffering promptly. The mice will be individually monitored using a clinical health score table for distress post-myocardial infarction surgery. When an individual score is reached or if a cumulative score is reached, animals will be killed humanely.
- Where tissues are required at very short time points post-myocardial infarction mice will not be recovered from deep anaesthetised and so experiences no discomfort.
- In the early compensated stages of heart failure animals are characteristically well and move freely. Deterioration of the heart is accompanied by specific symptoms. Regular

observation of the animals allows these symptoms to be used as humane endpoints. As with man, some deaths are sudden and presumed to be arrhythmic. When it has been possible to observe these, death occurs within minutes and loss of consciousness is likely to be rapid so that suffering is not prolonged. Telemetry systems will be used to assess arrhythmic (abnormality of the heart's rhythm) events.

- Anaesthetics will be used for surgery and for restraint post surgery (to limit pain/harm to the mouse when picked up and restrained post cardiac surgery). Pain relief medication will be given as necessary after surgery. Since rodents frequently do not show signs of pain, we will administer pain relief medication pre-emptively or longer if required, without waiting for clear signs. Animals will be regularly monitored so as not to exceed pre-determined endpoints that might increase the amount of distress caused.
- Flavoured drinks (e.g. Nesquick), spreads or jelly will be used to increase substance (pain medication, gene activating drugs) palatably.
- We will employ non-invasive imaging to acquire detailed scientific information with the least suffering caused to animals.
- Where possible we will perform two or more procedures under the same anaesthetic.
- Animals are housed according to the best recommendations and enrichment, and nesting material will be added to cages and where possible mice will not be singly housed. We will ensure there is acclimatisation to handling and procedures and optimal handling and interaction with the animals to maximise their welfare.
- All procedures will be continually evaluated, reviewed and refined to minimise and reduce experimental duration, animal numbers and suffering while maintaining or improving scientific benefits. To facilitate this, we will have regular meetings within the lab after every series of experiments, and we will also have similar discussions with our close collaborators.

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

We will refer to LASA guidance for surgery and aseptic techniques https://www.lasa.co.uk/current_publications/.

We will use guidelines (PREPARE) prior to initiating any experimental study to aid in the planning of each stage (https://norecopa.no/PREPARE), and guidelines (ARRIVE) to help in the design, analysis and reporting of all studies (www.arriveguidelines.org).

We will follow best practice guidelines for experimental models of myocardial infarction (https://doi.org/10.1152/ajpheart.00335.2017).

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

We will regularly consult websites such as Norecopa (https://norecopa.no/) and NC3Rs (https://www.nc3rs.org.uk/) and read the NC3Rs e-newsletter. We will take advice from dedicated technicians within the animal units.

**A Retrospective assessment of Refinement will be due by 12 August 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. Ageing and therapy in mouse models for neurodegenerative 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

**Key words**

Ageing and neurodegeneration, Therapy, aSynuclein pathology, tau pathology, gut

| 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 aim is to understand the processes that lead to nerve cell death in mouse models of Parkinson's and Alzheimer's disease and test interventions at early stages that decrease the severity of the disease and help the development of treatments

**A Retrospective assessment of these aims will be due by 02 August 2026**

**The PPL holder will be required to disclose:**

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

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

**Why is it important to undertake this work?**

Our findings will enable us to identify pathological pathways leading to abnormal aSynuclein and tau accumulation, the proteins that cause dementia in neurodegenerative diseases like Parkinson's and Alzheimer's disease. Key steps in these pathways may serve as potential targets for therapeutic intervention.

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

Diseases that kill nerve cells and lead to dementia, like Alzheimer's disease (AD), or great physical and mental disability, like Parkinson's disease (PD), have no cure. Treatments are being sought all the time but for the most effective treatments we require an understanding of the core processes that cause the disease. We are trying to understand the earliest processes that go wrong because revealing how they work may point to new therapeutic targets that delay disease onset so that people can lead a healthy life for longer. By genetic manipulayion, we have created unique mice that develop core features of these diseases that are also found in humans. One line of mice develops abnormal protein clumps (aggregates) made up of tau, the protein core of toxic fibres known as neurofibrillary tangles in Alzheimer's disease. Clumps of tau also cause other dementias, such as frontotemporal dementia, progressive nuclear palsy, and chronic traumatic encephalopathy, which develops in sports people exposed to repeated head impacts. The group of diseases that are caused by tau are knonw as tauopathies. Other lines of mice develop abnormal clumping of the protein alpha-synuclein, which forms the core of toxic Lewy Bodies in Parkinson's disease and similar dementias, known as alpha-synucleopathies. At the end of this project we will have acquired important new insights into how the abnormal protein clumps made of tau or alpha-synuclein are formed, and why they cause the death of nerve cells in the brain. We will also have determined how neuronal tau clumping causes insults to the surrounding supportive cells (known as glia) and how this leads to inflammation. Inflammation is a key aspect of both AD and PD that has been neglected until recently but now is a basic part of disease diagnosis. It has been proposed that PD begins in the gut and spreads into the brain through nerves that connect the two tissues. To test this hypothesis, we have created further genetically modified mice in which alpha-synuclein clumps begin forming in the gut, and eventually spread into the brain. By studying these mice, we will also understand the steps that lead alpha-synuclein to spread from the gut into the brain, and identify which species of the synuclein clumps are responsible for disease spreading and for the different symptoms seen in PD. We expect to test new compounds that inhibit alpha- synuclein aggregation into clumps and spreading to delay the development of pathology.

Understanding of these processes may also benefit clinical diagnosis identifying new participants in the disease process and by identifying molecules in body fluids that can be used for early detection of the disease. Our findings will be made publicly available by

publishing in open access journals, presenting our findings at open meetings, by collaborating with colleagues who use techniques that complement our range of methods that can add value to our findings, and with the pharmaceutical industry.

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

In the short term the research community will benefit from our outputs, by having new insights into mechanisms of disease progression and the molecules that can be targeted for novel therapeutic strategies. Through our work, we will have contributed to long-term societal benefits by improving the quality of life and well-being of the ageing population, since age is the highest risk factor for development of dementias and loss of mental functions. Our research will also benefit younger patients with inherited early onset diseases due to mutations in tau and alpha-synuclein that are incorporated in our genetically modified mice.

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

Outcomes of our research, including unsuccessful approaches, will be reported in conferences and webinars and in open-access, publicly available databases.

Several collaborations are in place and will be developed to obtain new tools to further the understanding of these pathological processes.

**Species and numbers of animals expected to be used**

- Mice: 5300

## Predicted harms

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

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

One out of six individuals over 80 years old will develop some form of dementia (the inability to think and remember) like Parkinson's (PD) or Alzheimer's disease (AD) and, to date, there is a lack of treatments that effectively stop or delay disease worsening. These diseases share in common the abnormal clumping of proteins such as aSynuclein (PD) or tau (AD), which become toxic and ultimately lead to the death of brain cells, which is the cause of dementia and movement problems. In humans, diseases that involve tau and aSynuclein start many years before the disease reveals itself as an impairment. These hidden stages of disease are not well understood but it is thought that if they could be diagnosed at an early stage before clumping there is better chance of effective therapy. For the past 20 years, our lab has produced several genetically modified mouse models that develop clumping of aSynuclein and tau that replicate key aspects of human disease, such as loss of neuron cross-talk, death of neurons, movement impairment and memory loss. By studying how the disease develops throughout the lifetime of the mice we will acquire new insights into what causes these proteins to clump and become toxic. These

insights will enable the study of the effect of potential cures that delay the beginning of these diseases and promote a healthy ageing.

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

Most of the PD animal models used in the studies covered by this licence will be aged until defined time-points (6-12-18-24 months) which correspond to different stages of disease; tissue from these animals may be used to study the pathways that lead to protein clumping. Brain fluid may be sampled once surgically using an implanted cannula, and blood may be sampled via the tail vein, to see if we can detect molecules that could be used to predict the disease in humans before it fully develops. The behaviour of mice will also be tested in some instances to see if they have developed a movement problem and whether their memory is impaired. At each time point, some mice will be killed under anaesthesia and tissues preserved so that the molecules identified can be matched with disease progression.

Once we have mapped how these molecules and behaviours appear during the aging process, some mice may be given drugs or treatments at early stages that may slow the clumping of aSynuclein and therefore delay disease progression. To monitor the effects of these treatments, the mice will be assessed using the same tests that we use in untreated mice, as described above. These treatments could potentially lead to new cures.

One idea is that PD develops first with aSynuclein in the nerves that line the gut, mainly the intestine (PD patients are often constipated) that then spreads to the brain. Mice with aSynuclein clumps in the gut will be tested for their gut function by giving them a small amount of a natural dye through a flexible tube inserted into the stomach and measuring the time for the dyed stool to be expelled during 4 hours. This experiment may be repeated in different groups of animals at 3, 6, 12 and 16 months. At each time point, some mice will be killed under anaesthesia and tissues preserved so the whole route that the clumps follow from the gut to the brain will be analysed.

Regarding PD beginning in the gut, it is suggested that aSynuclein clumping is caused by an irritation, for example a high-fat diet or inflammation. In another series of experiments, we will model such an irritation by, for example, feeding the mice with a high-fat diet. The same behavioural and gut function tests and endpoints will be applied as described above.

In certain instances, mice will undergo one session of sampling of the brain chemical dopamine, because the neurons that produce dopamine are the ones that die in PD and cause the disease. In this experiment, a small needle is implanted into the brain a day before, and we let the mice recover. The next day, a small amount (a few thousandths of a millilitre) of brain fluid is collected over 3 hours without causing any pain to the mice, after which the mice will be killed under anaesthesia. The fluid is analysed chemically for dopamine content and the brains will be analysed to determine disease stage.

In our tau models, the aim is to uncover the events that lead to the clumping of tau, and how tau causes cell death. We will also study why tau triggers the immune cells in the brain to cause inflammation. For this purpose mice will be killed at various stages of disease as determined by measuring the gradual loss of movement. The brains and spinal

cords will be analysed for molecules that change in the nerve cells and in the various types of immune cells that might be involved in the disease.

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

We do not expect adverse effects in the ageing protocols of PD animal models with regard to administration of a high fat diet or administration of therapeutic or labelling compounds. Animals undergoing surgical procedures will experience transient discomfort after surgery, however these animals will fully recover in less than 24 hours with no lasting effect on their general well being.

The tauopathy animal models will develop motor impairment beginning around 3-5 months of age and decline from there until the end point, which is 15% weight loss, or loss of movement of either hind limb, but with no pain or other signs of discomfort. Because of these symptoms, the protocol falls in the severe category. To study early stages of the disease, when some molecular events may start to take place with no impact on the motor system, we have set a moderate category. Animals under this protocol may have scruffy fur or a hunched posture but will otherwise lead a normal life, e.g. walk around the cage freely, associate with the other mice in the cage, and feed and drink normally.

All animals developing tumours as a result of advanced age will be humanely killed.

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

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

Mild 70%

Moderate 24%

Severe 6%

**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 02 August 2026**

**The PPL holder will be required to disclose:**

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

## Replacement

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

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

It is not currently possible to accurately re-create a functioning nervous system that mimics the complex interaction between different neuronal cell types, tissues and organs. Therefore, to understand the mechanisms behind disease progression and full therapeutic effectiveness of relevant drugs, an animal model is needed.

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

We are currently using cell culture technologies involving human cells that are allowing us to make great progress in understanding how aSynuclein and tau proteins are toxic to cells. This procedure involves taking human skin samples and converting the cells into cell types that reside in the brain in 2D culture systems. Currently, we are also generating organoid cultures that may help to further understand the involvement of blood supply, the immune cells in a 3D environment more closely resembling the complex cytoarchitecture of the human brain. We also use techniques that promote clumping of aSynuclein and tau in a test tube to test compounds that might intervene in this process but this also has limitations, as the clumps in the test tube do not accurately replicate of the clumps in the brain, and the cell responses that might be part of the toxic process is missing.

**Why were they not suitable?**

Cell culture technologies are very useful, however they complement but do not replace the study of animal models. It is impossible to recreate the 3D structure of the brain in a dish, given all its cell types, intraconnections, and influence of other tissues, most especially in the gut and the immune system.

**A Retrospective assessment of Replacement will be due by 02 August 2026**

**The PPL holder will be required to disclose**:

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

## Reduction

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

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

We have several years experience in using these models of synucleinopathy and tauopathy. We have been reducing animal number year on year as we learn more about the models and understand the disease process better. The number of animals is kept to

the minimum by carefully monitoring the colony size and breeding, and matching these to the demands of the experiments. By applying statistics to the experimental design, we will obtain meaningful results that will prevent the need for experiment repetitions.

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

For best experimental design we refered to the PREPARE guidelines checklist: https://norecopa.no/prepare/prepare-checklist, which considers the steps needed to ensure that meaningful results are obtained while using the minimum number of animals. Taking these into consideration, we carefully weighed the balance between harm and benefit based on several years of experience in using our models of synucleinopathy and tauopathy. We have been continuously improving our understanding of how the animals respond during the development of the disease process, allowing us to reduce animal use year on year through careful maintenance of colony size and breeding. By applying statistics to the experimental design, we ensured that we will obtain meaningful results that will prevent the need for experiment repetitions.

**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 coordinate and share tissues between multiple users and generate a tissue bank from animals, maximizing the use of each mouse. For some experiments, we will culture cells or brain slices from the mice, generating multiple samples from a single mouse.

For the majority of the experiments, preliminary (pilot) studies have already been conducted previously so will not be needed in this licence and data from these pilot studies will be used to inform work undertaken on this project.

For gut inflammation experiments, we will conduct a pilot study on a small cohort of animals to establish the technique based on previously published data in our transgenic model.

**A Retrospective assessment of Reduction will be due by 02 August 2026**

**The PPL holder will be required to disclose:**

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

## Refinement

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

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

We will use transgenic mice that model the most common neurodegenerative diseases, Parkinson's disease and Alzheimer's disease. Parkinson's disease is modelled by expression of the human protein alpha-synuclein in a form that clumps in nerve cells and causes their death; Alzheimer's disease is modelled by expression of mutant forms of the human protein tau, which forms clumps and tangles that cause nerve cell death and brain inflammation. We will take tissues from these animals after they are killed at different stages of the disease to study how the disease develops. In some cases animals will be treated with drugs and their brain function monitored under analgesia. When animals are used in an invasive, potentially painful procedure, analgesia and anaesthesia will be used to minimize suffering. Extra monitoring will be provided to those animals for at least 24h after the procedure and under close supervision of the establishment veterinary surgeon. Regarding embryo transfer methods, we hope to explore the non-surgical embryos transfer (NSET) method as a refinement over the course of the licence.

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

We are using mice because they have a nervous system that is sufficiently similar to that of humans and where the biology of neurodegeneration is almost the same. In addition, mouse behaviour is well characterized, and our mouse models mimic the behaviour in the human clinical condition.

Furthermore, mice can be genetically manipulated, allowing molecular hypotheses about the mechanism of disease development to be tested.

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

We will continue to enrich the environment with nesting and tunnels. Induction of inflammation by diet change instead of chemically induced inflammation is a more refined and physiologically relevant method. As we come to understand the later stages of pathology, this will guide us as to what to look for at earlier stages of the disease, thus avoiding the development of the most severe clinical disease.

We have developed a traffic light scoring system to track the development of disease progression in one of our mouse models which we use to determine when the mice need mash and gel and softer nesting material to offset the increased disability. By weighing the mouse every day when they reach 10% weight loss we can more precisely define the humane end points. We will remove males from pregnant P301ST43 females to ensure that each female has only one litter so that she remains healthy during pregnancy and can take care of her litter until weaning.

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

We will follow the NC3Rs guidance notes and webinars (https://www.nc3rs.org.uk/welfare-assessment) and those from the Jackson Laboratories (https://www.jax.org/news-and-insights/jax- blog/2016/march/experimental-design-top-four-strategies-for-reproducible-mouse-research). Advice and notes provided by the LASA guidelines will be also consulted (www.lasa.co.uk/publications.html) and we will follow the ARRIVE guidlelines (Animal Research: Reporting of In Vivo Experiments). The guidelines provide a checklist of recommendations to improve the reporting of research involving animals to maximise the quality and reliability of published research, and enable others to better scrutinise, evaluate and reproduce it.

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

We will continue to routinely access the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) website for information and advice and spread this knowledge among all members of our group. We also follow Norway's 3R centre (NORECOPA) guidelines (https://norecopa.no/about-norecopa). For example, using a tunnel to pick up mice initially to reduce anxiety because picking up by the tail enables a hind limb clasping test without causing anxiety. (https://www.nc3rs.org.uk/mouse-handling-webinar). We will also refer to the RSPCA website (https://science.rspcs.org.uk/sciencegroup/researchanimals) for further information.

**A Retrospective assessment of Refinement will be due by 02 August 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?

# 10. Immune responses to infection and during inflammation

**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
- 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**

Virology, Immunology, Vector biology, Inflammation, Dermatology

| Animal types | Life stages |
|---|---|
| Mice | neonate, juvenile, adult, pregnant, aged |

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is 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
- Required at inspector’s discretion

## Objectives and benefits

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

**What’s the aim of this project?**

This project will study mammalian host immune responses in the skin to define new therapeutic targets. We have a particular interest in host responses following infection with virus, focusing on responses to arthropod-borne virus and arthropod-derived factors. Comparisons to chronic inflammatory diseases that involve similar biological processes will also be made.

**A Retrospective assessment of these aims will be due by 02 August 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Virus spread by arthropods, called arboviruses, are a large group of emerging, medically important pathogens for both humans and economically important livestock. There exist few medicines or vaccines to treat and/or prevent infection with arboviruses. There is a clear unmet need to better understand how these viruses cause disease, and new studies that identify new medicines is urgently required. This project will define key aspects of infection that will enable us to design new medicines. In addition, the underlying biological responses that underlie antiviral immune responses are also dysfunctional in other skin diseases such as psoriasis and systemic sclerosis. Therefore this work will aid our understanding of immune responses to both acute virus infection and chronic inflammatory diseases.

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

By studying the fundamental mechanisms underpinning these processes we can better understand the complex interplay between mammals, viruses and their mosquito vectors and in doing so will help us to;

- define new mechanisms of innate immune function during the early events of an important group of emerging infectious disease
- identify the most relevant aspects for disease control and prevention
- identify mosquito-derived factors in their saliva that modulate mammalian host susceptibility to virus infection
- identify putative biomarkers to aid diagnosis and risk stratification of infected patients
- identify commonalities in skin responses to those that cause inflammatory skin diseases, such as psoriasis provide a highly relevant model system for studying innate immune function that together will be of interest to a wide community of scientists, clinicians, veterinarians and public health professionals

The threat posed by emerging infectious disease to human health and the economy is substantial. It is important, more now than ever, that we better understand these diseases so that we can develop new treatments and vaccines. On a global level, one of the most important groups of infectious diseases are those that are spread by biting mosquitoes. This includes diseases caused by viruses, such as dengue, zika and chikungunya. Most such infections are found in warm climates. However, due to the worsening climate

emergency and increasing globalisation, the areas affected by these diseases has increased at an alarming rate. Today, over one third of the global population now live in areas at risk of outbreaks.

The timing and emergence of these epidemics is almost impossible to predict. We suggest that one aspect that can increase our preparation for such outbreaks is a better understanding by which our body defends themselves against virus. This will enable us to design new therapies that are useful against a broad range of these viruses.

Infection with mosquito-borne viruses most often causes a debilitating flu-like illness, which can be associated with severe complications and sometimes even death. This combined with a lack of anti- viral medicines makes it difficult for clinicians to treat and manage patients. In addition, symptoms during the early stages of diseases are similar, irrespective of the virus they are infected with, making a timely diagnosis difficult. Therefore, new medicines that target common aspects of infection, irrespective of the causative virus, have the potential to transform the treatment of patients.

This proposal aims to ultimately reduce the burden of these diseases by increasing our understanding of the skin's immune defence to infection at the mosquito bite. When mosquitoes bite people they inject virus into the skin. This is a key stage during which the virus infects cells in the skin and replicates, and is common to all mosquito-borne virus infections. Following infection at the bite, the body's immune response is activated, which if sufficiently robust can hinder the virus from replicating and causing disease. We have recently found that the strength and type of immune responses activated at the mosquito bite can influence how bad the infection becomes in the rest of the body. In particular, we found that a component of immune responses, interferon, is too slow and not sufficiently robust at the mosquito bite to stop the virus. This allows the virus to replicate and spread around the body and cause disease. It is not clear why interferon is not being activated properly. Interestingly, we have recently shown that a skin-applied cream that contains an interferon booster, can dramatically increase the skin's resistance to virus infection. This showed that it is possible to therapeutically activate the body's own immune responses at the mosquito bite to alter susceptibility to a wide spectrum of these diseases.

How the interferon response to virus is coordinated by the body at mosquito bites is not well understood. If we want to better design new therapies, we need to know how these immune responses in the skin are organized at the cellular and molecular level. This project will bring together distinct scientific and clinical expertise to transform our understanding immune defence at mosquito bites. In doing so, we will identify new ways to treat and prevent infection.

The role that mosquito derived salivary factors have in modulating infection at the mosquito bite is also not well defined. We will specifically define what role mosquito saliva has in modulating host susceptibility to virus infection. We have already identified one such mosquito salivary factor that increases blood vessel permeability and thereby enhances skin infection with virus. In this project, we define the mechanism behind this observation and additionally seek to define other mosquito derived salivary factors that similarly

influence virus infection. Identification of these factors may enable the development of vaccines that target them.

As such, this work has the potential for publication in the very highest impact multidisciplinary journals. We also believe this work is likely to lead to productive new collaborations with industrial and clinical groups that will translate our basic science discoveries into real world impact.

The overall benefit will be to enhance our understanding of immune and inflammatory responses, although where possible we will make use of these findings as best we can by working with pharmaceutical companies and clinicians to develop new medicines and/or strategies to help treat these diseases.

Thus, this project will combine expertise from the distinct disciplines of immunology, virology and dermatology in an imaginative and complementary manner, the results of which will likely benefit a range of academic and clinical disciplines. Within the academic sphere, this work will generate significant and important new immunology and virology insights at a key, but understudied, stage of infection with an increasingly important group of pathogens.

This project provides a unique opportunity to fuse distinct expertise and resources in a novel way that will lead to new academic and translational research opportunities. Thus, by combining the strengths of our ‘underpinning’ animal and human-based studies, with the translational/clinical approaches of our dermatology colleagues, this project will be uniquely placed to both build capacity for future research and in doing so determine the relevance of early skin immune responses on disease severity.

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

Academic and associated beneficiaries

This project will make use of our existing collaborations that have been highly successful in generating key (now published) insights on skin arbovirus infection.

We are ideally placed to now extend this collaborative network to include relevant bioinformatic expertise. Together, this will generate a unique new collaborative relationship that combines expertise in; dermatology; molecular arbovirology; mosquito biology; and bioinformatics/transcriptome analyses. This will be integrated by our team’s existing, complementary expertise in inflammation biology and arbovirus pathogenesis to generate insights of interest to wide number of disciplines. Additionally, our team includes a new PhD student who soon begins a PhD project studying skin patient (psoriasis and eczema) susceptibility to arbovirus. These studies will gain considerable advantage and synergy from the work supported in this proposal, including use of human skin biopsies for explant infection.

In summary, due to the broad-based nature of the proposed work, the wider academic beneficiaries will include a spectrum of virologists, immunologists, translational medical dermatology and infectious disease clinicians.

Longer term non-academic beneficiaries

Our work is highly translational and has great potential for informing the design of new strategies for alleviating the health burden of e.g. mosquito-borne viruses. Based on the findings of our work, we anticipate working with colleagues in clinical medicine and industry to formulate new approaches for the design of medicines and vaccines.

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

This collaborative project will maximise the benefit of work to the institutes and individual research groups involved in several distinct ways;

- there will be expertise transfer between our groups in the areas described above and capability building between them. Dermatology-based academics will be provided with the opportunity to gain expertise in basic biology-based approaches, including in vivo models of infectious disease. In addition, our group will gain access to the clinical and patient resources of our dermatology colleagues.
- as such, this collaboration will provide opportunities for researchers and students in each group to gain new research experiences and skills that will facilitate and enhance their research career development
- this project will also allow our clinical co-investigators to widen the scope of their research portfolios to now also include studying infectious diseases of global importance that particularly affect low and middle-income countries.

We expect to publish the results of this work as several publications in internationally-leading multidisciplinary journals, and additionally, present this work at national and international conferences. To increase the efficiency by which we disseminate our findings through these routes we will also pre- publish our data on BioRxiv. Using this real time feedback approach, we can rapidly disseminate our key findings to the international community. We also have a successful track record of engaging the academic and wider scientific community through a combination of social media. Explaining the importance of scientific research to the wider society is also necessary if we are to continue receiving public support for our research and because we want to promote science as a rewarding career. Public engagement requires adequate and sustained discourse that is communicated in manner appropriate for the target audience. We will target non-specialist audiences using a combination of; online social media; coverage in traditional media outlets; our website and through face-to-face interaction in both the laboratory (e.g. with visiting school students); and in public environments.

**Species and numbers of animals expected to be used**

Mice: Our studies exclusively use mice and we anticipate using up to 5,000 mice over the 5-year timeframe of this project. Note that 1200 mice from protocol 1 will be transferred (in

continuous use) in other protocols, so that the total mice used will not exceed 5,000. 300 of the mice in protocol 1 will be used as breeders to maintain transgenic lines.

## Predicted harms

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

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

The mouse is the species of choice as it can be genetically manipulated to alter gene function in ways that are not currently possible using other mammalian species. In addition numerous reagents are available for examining, and intervening in, immune and inflammatory responses in mouse models.

We will study the responses of young adult mice as mice at this age best represent human responses.

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

The primary purpose of this project is to understand the body's response to infection with viruses and the inflammation this causes. Therefore, mice will be injected with virus in the skin, along with agents that we hypothesise may influence susceptibility to infection. This will be key for identifying new medicines to treat these diseases. As we are mainly interested in mosquito-borne virus infections, such as Zika virus, we will also allow mosquitoes to bite mice either before virus inoculation. We will use a variety of advanced techniques to describe the course of infection such as imaging and blood sampling. Where possible we will always use techniques that have lowest possible impact on animal welfare.

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

The vast majority of experiments will only result in mild and very transient suffering, such as injection with a needle. We use custom made needles that are exceptionally thin, that we believe cause far less pain than conventional needles. We also use temporary anaesthesia that puts the mice to sleep for a few minutes - this can be done if injection will occur in a sensitive part of the skin such as the foot.

Therefore, we minimise any pain or discomfort that may be generated from the injection. If allowed to progress, the virus infection will lead to brain inflammation, encephalitis, which develops very rapidly. This most commonly presents as limb paralysis along with easily detectable sickness behaviour. As soon as these clinical signs of brain infection are detected, the mice will be humanely culled. Therefore, the total time that the mouse becomes unwell at this later stage of infection will not be more than a few hours.

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

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

The majority of mice (estimated at >90%) will only experience mild suffering, as we will humanely cull the mice prior to the development of more severe disease caused by the virus. However, to study the later stages of diseases and e.g. to determine what effect any new medicines we develop have, we will let some mice progress to more severe disease. The total length of time that a mouse will experience severe suffering will be very brief, as mice tend to develop signs of suffering within a few hours, at which point they will be immediately and humanely culled. This is essential as we can determine whether new medicines are working to stop the disease.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 02 August 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

This project will seek to understand the complex biological interaction between biting mosquitoes, the viruses that they carry and their mammalian hosts. We have evidence that mosquito bite inflammation is highly counterproductive and helps viruses establish infection in the skin. This project will work out how the immune system responds to mosquito bites and how viruses spread from bite sites to the blood and other tissues. This will be useful to scientists, health professionals, drug companies and policy makers who shape our response to these epidemics.

Because of their complexity, the immune and inflammatory responses to infection at mosquito bites, it is necessary for us to use mouse models. This is because immune responses involving numerous different cell types and molecules in ways that cannot be yet replicated outside of an animal. This is because immune responses are carefully orchestrated in an intact animal in ways that cannot be recapitulated using non-animal alternatives.

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

We will be employing and using a range of non-animal alternatives that include in vitro cell culture. This includes culture of skin cells, leukocytes and endothelial cells alone and in co-culture to better mimic the microenvironment of the skin.

**Why were they not suitable?**

These non-animal approaches will help us to reduce the number of mice used considerably. However, if we are to prove that a particular molecule or cell is important and can be targeted therapeutically, we must also undertake some experiments using mice.

**A Retrospective assessment of Replacement will be due by 02 August 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 more variation there is in the data generated by an experiment, the more animals are required to statistically prove or disprove a result. Based on our published studies we have a good idea of the variation we expect to see in our results. Using this variation, we are able to use statistical methods to calculate how many mice we would need in future experiments. This was undertaken with the help from professional statisticians.

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

We have many years' experience of working with animal experimentation and have developed robust protocols involving the minimum use of animals required to provide statistically significant analysis.

We also obtain advice from statistical analysis colleagues regarding the design of new experiments.

We have also made use of the Experimental Design Assistant (EDA). This is a free online tool from the NC3Rs, designed to guide researchers through the design of their experiments, helping to ensure that they use the minimum number of animals consistent with their scientific objectives, methods to reduce subjective bias, and appropriate statistical analysis.

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

At all times mice will be bred in the most efficient manner possible, with regular communication between the animal welfare officers, the animal house technicians, the personal licence holder and the project licence holder. Regular team meetings once a week will include a dedicated section that will discuss the optimum use of animals. We have devised creative and resourceful ways to maximise the use of animals for example suing multiple tissue from one animal for different projects - such as using bone marrow to generate macrophage cultures, while also using the skin for other experiments.

Furthermore, where necessary we will undertake pilot studies that use only a very few mice. This will generate data that can be used to predict what numbers of mice would be required to prove or disprove the hypothesis.

In this way we can greatly reduce the number of animals used.

**A Retrospective assessment of Reduction will be due by 02 August 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

We will be infecting mice with viruses that cause great harm to human health on a global scale. It is inevitable that this will cause some suffering to the mice. The severity of the procedures used will be kept to a minimum, whilst providing meaningful data for translation of these approaches to patient care. For example, we will prevent the majority (more than 90%) of these mice from experiencing anything more than a limited and mild form of suffering. This might include e.g. a needle inoculation. Suffering will be limited by humanely culling the mice at an early stage of infection, at which point the mice do not perceive any pain or discomfort from the infection.

For some mice, to better understand how these viruses cause disease and how new medicines could treat these diseases, we will let ust a few of the mice progress to later stage of infection. This will cause suffering of the mice to be more than mild, but will be short in duration. This is because we will use a virus that only causes suffering for just a

short few hours. Regular monitoring of mice will ensure that mice are immediately and humanely culled to prevent unnecessary suffering. By doing this we will help uncover important new insights into disease and new medicine to help treat them.

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

The mouse is the species of choice and it can be genetically manipulated to alter gene function in ways that are not currently possible using other mammalian species. In addition numerous reagents are available for examining, and intervening in, immune and inflammatory responses in mouse models.

Furthermore, mice constitute the 'lowest' mammal recognised to be relevant for immune and inflammatory disease studies.

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

To minimise harm to animals, especially those on procedures with which we have less experience, animals will be monitored regularly for routine signs of ill health or distress. Anaesthetics will be used as appropriate to the procedure being undertaken and advice from local veterinary surgeons will be sought in any situation where animals are showing unpredictable signs of ill health or suffering.

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

Discomfort and distress experienced by the animals will be limited to unavoidable procedures required for the conduct of sound research.

General Guidelines

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NCRs) website (https://nc3rs.org.uk//results-search/all/Refinement) is a rich resource for obtaining new approaches for refining animal-based research, with regular updates and evidenced guidance supplied. This published online resource will be consulted with in a continual manner to ensure our experiments are undertaken in an optimally refined manner. The NCR have published guidance that we will use to guide our studies;

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

In addition, general guidelines on how to conduct experiments in the most refined way, has been published by the NC3Rs in collaboration with the MRC and other funding bodies. This document provides general guidance to researchers and associated veterinary and animal care staff using vertebrates in bioscience research.

https://www.nc3rs.org.uk/sites/default/files/Responsibility%20in%20the%20use%20of%20animals%20i n%20bioscience%20research%20-%20July%202015.pdf

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

We will stay informed in all aspects of the 3Rs using a number of local, national and international resources and practices. Locally, this includes discussing the principles and practice of 3R at dedicated sessions during our weekly team meetings. This will be supplemented with monthly discussion at our local animal house meetings, during which all users can participate and engage in new practices that aim to enhance our procedures and promote the 3Rs. There are also regular postings by email from our institute, in which opportunities to gain training in 3Rs is made available. These will also be promoted i our team meetings. National and international resources will also inform our experimental strategy, with the ultimate aim of employing the principles of 3Rs wherever possible. Such resources include the website for the NC3R. The NCR3 is a UK-based scientific organisation dedicated to replacing, refining and reducing the use of animals in research and testing. Their website provides an extensive library of 3Rs resources. This includes guidelines, practical information and themed hubs. Links to publications, other online resources, and video and training materials are also provided and cab found at; https://www.nc3rs.org.uk/3rs-resources

**A Retrospective assessment of Refinement will be due by 02 August 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?

# 11. Pathogenesis and therapy of vascular 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

**Key words**

Cardiovascular disease, Vascular disease, Disease aetiology and pathogenesis, Stem cell, Disease intervention and prevention

| 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 overall aim of the present project is to investigate novel underlying causes of vascular diseases, and to test new therapeutics for these diseases.

**A Retrospective assessment of these aims will be due by 16 September 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Cardiovascular disease (CVD) the disease affecting heart and blood vessels in human body. It is a leading cause of the death in humans, in which arteriosclerosis (narrowing of the blood vessels)-induced vessel blockage is the underlying problem. Arteriosclerosis is a severe pathological condition that underlies several important adverse vascular diseases including coronary artery disease (CAD, causing heart attack), stroke, and peripheral arterial disease (PAD), responsible for over 40% of all deaths in Western countries. Atherosclerosis is an inflammatory disease, in which risk factors such as hyperlipidaemia, hypertension, diabetes, smoking and infections can directly or indirectly stimulate the arterial endothelium, resulting in its dysfunction, damage or both. Once the integrity of the endothelium is interrupted, lipid penetration and mononuclear-cell adhesion might be initiated. Although great efforts have been put into research to understand the development and progression of atherosclerosis in the past decades, the underlying causes of this disease development and progression are not fully elucidated.

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

From these experiments proposed in the present project, we expect to identify some of the cellular and molecular mechanisms underlying the formation and development of atherosclerotic lesion, aortic aneurysm (abnormal expansion of the aorta)/dissection (tearing and rupturing of the aorta), and/or vascular calcification (bone-like aorta), which provide the basic information for design of a new drug to target the novel molecules to prevent these diseases progression by enhancing blood vessel repairing after damage. Additionally, when we know the contribution of stem cells to vascular diseases, we can develop a way to promote stem cell differentiation toward vascular cells to repair damaged vessels. Finally, we will test the therapeutic effects of mouse and/or human stem cells or the tissue-engineered vessels generated from stem cells in cardiovascular diseases by using the murine model for human cell transplantation or tissue-engineered vascular grafting. Thus our results will provide basic knowledge useful for both scientific research and clinic application in the future in cardiovascular diseases, e.g. preventing blood vessel blockage and creating a tissue-engineered blood vessel.

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

A series of experiments will be performed to address the underlying causes of vascular cell death, proliferation, differentiation and vascular regeneration. These studies will provide us potential targets to design new drugs to enhance cell survival and vascular regeneration. Once the contribution of stem cells to arteriosclerosis is confirmed, we can find out a way to promote stem cell differentiation toward vascular cells to repair damaged vessels. Finally, we have initially tested a tissue-engineered vessel in a murine model, and we will produce a second generation of tissue-engineered vessel grafts, which will be tested in animal models. Thus, our results will provide basic knowledge useful for both

scientific research and clinical application in the future in cardiovascular diseases, e.g. new drug discovery, preventing blood vessel blockage, and generating the next generation of a tissue-engineered vessel.

Apart from the abovementioned medical progress and uncovering novel therapeutics for human diseases, the additional likely beneficiaries of the proposed project include both academic and non- academic beneficiaries. Specifically, the postdoctoral research associates, PhD students, and those actively involved in this project including other researchers and post-/under-graduates will be primary beneficiaries, through gaining expertise in medical research. Moreover, the new knowledge in relation to the possible functions & therapeutic potentials of the studied genes/proteins/cells/agents in vascular disease will likely immediate benefit to individual academics and academic research groups working in closely related fields of scientific research. More specifically, researchers interested in cardiovascular science or vascular biology will benefit from the identification and characterization of candidate genes/proteins/cells/agents for preventing and/or treating vascular diseases. Researchers interested in molecular & cell biology, proteinase biology, vascular biology and cardiovascular medicine may benefit from better understanding with regard to the functional impacts and involvements of the studied genes/proteins/cells/agents in vascular disease.

By uncovering the importance of the studied genes/proteins/cells in vascular disease and exploring the therapeutic effects of the local/systemic delivery of small molecule inhibitors/activators/agents that target these identified signalling on vascular diseases, the findings obtained from this project could provide new targets or strategies for future therapeutic intervention in these lethal aortic disorders. This project is therefore likely to benefit those academics working in the field of vascular therapeutics and the researchers working in the drug companies who are interested in developing novel therapies for human diseases including cardiovascular diseases, which eventually benefit to patients with these diseases.

Research performed as part of this project proposal will be submitted for publication in the leading peer reviewed international journals that will benefit to all the academic and non-academic beneficiaries within UK and globally. Additionally, we will share data with collaborators and present our results pre- and post-publication to colleagues at meetings and conferences on a broad section of disciplines. The dissemination of our research data in the national and international conferences will provide ample opportunity for us to interaction with all levels of the worldwide academic communities.

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

Written communication ensures the longevity of our work and its availability forever. Dissemination of results to both the scientific community and clinicians will occur primarily through peer reviewed scientific publications in high-visibility journals with open access and submission of abstracts to national and international conferences as well as via invited reviews and presentations. Institutional Press and Research Office have excellent facilities for disseminating scientific information to members of the public through a variety of different media and forums. The research funders and the institutional Press office will

always be contacted in advance of publication for any advice on public or media engagements.

Our institution also provide regular departmental meetings, institute seminars and lecturer series to share our research with scientists and clinicians across arrange of disciplines and research backgrounds. Each year we will hold a wide range of school-wide scientific meeting including annual research review and conference, all of them will showcase the cutting edge of the research undertaken within medical school. We have been invited/selected to present our recent research findings in all these conferences, and play an active role in contributing to the successful of these meetings.

We anticipate that the findings obtained from this study will also be presented at key international scientific conferences. These organisations regularly make public press releases concerning research communicated at the conferences, which will further maximise the academic and scientific impact of our research. We have been frequently invited/selected to present our research findings in all these conferences, and play an active role in contributing to the successful of these meetings.

In liaison with institutional research office and research funders, the research findings will be presented to wider lay audiences. With one-third of all deaths still attributable to cardiovascular diseases, the research outlined in this proposal will attract a wide public interest. Our institution actively supports the wider dissemination of scientific research through hosting a wide range of outreach engagements to support its student and staff, and holding annual school open days, public lectures and workshops within different research centres. We will also engage with children in the local community through involvement with local Science Festival. Each year, our festival attracts hundreds of local students from primary schools to sixth forms.

**Species and numbers of animals expected to be used**

- Mice: 2600

## 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 lowest vertebrate group, which have a relatively well-defined genetic map. There are a large number of strains of genetically altered mice (in which one or more of their DNAs/genes were added, removed, or modified) available for study. Currently, genetically altered animals is the best scientific means to study the true contribution and causal effects of any specific gene to/in embryonic development as well as disease pathogenesis. Other advantages for using murine models are excellent availability of reagents, mouse antibodies, and mouse cell lines.

Genetically modified animals will be used because no other suitable tools exist to selectively and specifically manipulate the individual gene/protein that we want to study in vivo.

Most of the animals used in the study will be adult mice, with an exception of aortic dissection (an injury to the innermost layer of the aorta allows blood to flow between the layers of the aortic wall, forcing the layers apart) which uses mice at three to four week old. A drug called BAPN was used to induce aortic dissection in the young mice because this drug can inhibit an enzyme that plays a crucial role in maturation of elastin and collagen fibres within aorta, which mainly occurs between three to four weeks after birth. Thus, administration of the drug BAPN into fast-growing young animals (three-week old) is absolutely required to induce aortic dissection.

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

In a typical experiment, mice will undergo bone marrow transplantation (to study if a gene/protein in bone marrow cells has a function in vascular disease), receive an altered diet (e.g. high cholesterol), or an injection of a substance/agent that modify the blood cholesterol levels or can stimulate the development of vessel changes. The animals will or will not receive a surgery (e.g. injuring the inner layer of blood vessel, blocking the blood supply to tissues, or blood vessel transplantation). Some animals may be given further treatments with cells, genes, special growth factors, drugs, other agents, etc. during the surgery or at later time. Occasionally, some mice will undergo image scanning to visualize and monitor disease development/progression, as well as therapeutic effects of any treatment. At the end of these experiments and/or when signs of discomfort or pain are manifested, the animals will be humanely killed and tissues collect for biochemical and histological analysis.

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

Based on our experience, adverse effects caused by treatments are anticipated to be very limited in the most of our protocols and where they do occur to be very brief in duration. Although it is anticipated to be very rare, following adverse effects may occur in some animal after procedure: hunched posture, loss of appetite, weight loss, dehydration, diarrhoea, inflammation and infection, difficulty of breathing, or sudden death (due to aortic rupture). The potential adverse effects associated with these studies will be minimized by the use of anaesthesia, aseptic surgical procedures and use of painkillers. High standards of housing and care also minimize the stress associated with the procedures.

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

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

All the procedures described in this project are well tolerated by most of animals, but we expect that approximately 5-15% of animals on most protocols will show a mild to

moderate phenotype. Some animals (up to 30%) on protocol 5 (Aortic aneurysm/dissection) will show a severe phenotype. However their suffering will be very short duration due to sudden death caused by acute aortic rupture.

**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 16 September 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

The major aims of the present project are to study the problems behind vascular diseases and to discover new therapeutic methods for controlling and preventing these life threatening conditions. Currently, the best method available to address the potential effects of any gene and/or treatment on atherosclerosis and other vascular pathologies as detailed in this project is to compare atherosclerotic lesions and/or vascular disease development/progression from given gene deficient and wild-type animals with or without further therapeutic interventions. There is no suitable non-animal alternative to the approach to be used, as the development of vascular diseases, for instance, atherosclerosis (where the medium/large size of artery wall becomes harder and narrow) or aneurysm (a localized, blood-filled balloon-like bulge in the wall of a blood vessel) involves multiple cell types as well as many circulating factors that regulate the accumulation of these cells in atherosclerotic lesions, and there is no non- animal model available that  properly mimics this complex system.

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

In vitro cellular system and human aortic tissues from patients with vascular diseases.

**Why were they not suitable?**

Although in vitro cellular system serves as an excellent platform to study the signal pathways behind vascular pathologies, and the simplistic, cost-effective nature of in vitro setups had become well-integrated by most fields of biochemical research, yet many do agree unanimously to the fact that in vitro cultured cells differ tremendously from their tissue-level counterparts, therefore do not reflect normal cellular behaviours in their naïve

microenvironments under disease settings. More importantly, the in vivo complex interactions among different cells, neural/hormonal regulatory factors, and haemodynamic environments can't be truthfully replicated in vitro, and the whole system effects of any intervention/gene/protein on vascular diseases are difficult to model using these in vitro cellular systems.

Finally, although human aortic surgical specimens provide invaluable information about the pathogenesis of various vascular diseases, they are extremely rare, and mostly from advanced disease stages (end-stage of disease) with no comparable normal controls. Therefore, animal models described in this project stand as the most valuable tools for us to study the pathophysiology of vascular diseases.

**A Retrospective assessment of Replacement will be due by 16 September 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

There are three research objectives in this project, in which we estimates that approximately 2,600

mice in total will be used over five years, among them, roughly 2,000 are GA mice, which will be bred and maintained under objective-1. We estimated that 1,560 and 1040 mice will be used for objective-2 & -3, respectively, over five years.

Specifically, in Objective-2 we estimated that 260 mice will be used in spontaneous atherosclerosis for studying up to 10 genes/treatments (20 control/treating groups), 260 mice will be used in transplant arteriosclerosis (130 mice for donor/recipient mice, respectively) for studying up to 5 genes/treatments (10 control/treating groups), 520 mice will be used in injury/stenting-induced arterial remodeling protocols for studying up to 20 genes/treatments (40 control/treating groups), 260 mice for vascular calcification (10 genes/treatments, 20 control/treating groups), and another 260 mice for aortic aneurysm (AA) or dissection (AD) (10 genes/treatments, 20 control/treating groups).

In objective-3, roughly 260 mice will be used for spontaneous atherosclerosis (10 genes/treatments, 20 control/treating groups), 260 mice will be used for in vascular graft arteriosclerosis (130 mice for donor/recipient mice, respectively) (5 genes/treatments, 10 control/treating groups), 260 mice in injury/stenting-induced arterial remodelling studies

(10 genes/treatments, 20 control/treating groups), 130 mice for vascular calcification (5 genes/treatments, 10 control/treating groups), and another 130 mice for aortic aneurysm (AA) or dissection (AD) (5 genes/treatments, 10 control/treating groups).

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

Every single animal experiment will be designed with assistance from the Experimental Design Assistant (EDA), a free online tool from the NC3Rs (https://www.nc3rs.org.uk/experimental-design- assistant-eda). Apart from optimization of the experimental plan, EDA system will also provide excellent assistance in generating randomisation sequence, supporting for allocation concealment and blinding, as well as calculating group sample size. In doing so, all the animal experiments will be designed to ensure that the minimum number of animals with enough power to detect a statistically significant effect will be used in order to achieve the specific scientific objectives with high reliability and reproducibility, as well as least subjective bias before they are carried out. Apart from EDA, other methods (e.g., Research Randomizer, https://www.randomizer.org/) or software (Random Allocation Software) could be used to generate the randomisation sequences/numbers for any given experiment. Additional methods or tools (e.g., G*Power calculator: https://stats.idre.ucla.edu/other/gpower/, Sample Size Calculator: https://clincalc.com/stats/samplesize.aspx) could be used for sample size calculation. The researchers will be blinded to the group allocation at the different stages of the experiment. All of these measurements will ensure the minimum number of animals will be used under each objective.

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

We will conduct systematic literature reviews on similar studies since it provides more appropriate sources of information on variability. If that is impossible and resources are allowed, pilot experiments will be carried out in small number of animals to detect important information (such as effect size of treatment, variability) to assist power calculation in determining group sample size.

**A Retrospective assessment of Reduction will be due by 16 September 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

Mice are the species of animal with the lowest capacity to experience pain, suffering, distress or lasting harm, which have a relatively well-defined genetic map and can relatively easily be genetically altered. ApoE is a protein that responsible for transporting fats from blood cells to the liver, and thus removes blood fats. Mice without ApoE gene fed with the normal mouse food have spontaneous high level of blood fat and atherosclerosis (where the medium/large size of artery wall becomes harder and narrow) in the arterial wall, which are similar to those seen in humans. All the animal models chosen in this study are the least invasive and simplest of those available, which have been reproduced and reported by many other laboratories.

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

To elucidate the underlying causes of cardiovascular diseases (CVDs) and study any potential therapeutic effects of drugs, genes or stem cells on CVD prevention, in vivo systems approaches of CVDs would be essential. Although the Zebrafish model has been used for decades to study development and pathophysiology, a variety of drawbacks associated with Zebrafish models such as inadequate amounts of tissue for analysis, lacking of zebrafish-specific antibodies and zebrafish cell culture systems, anatomical difference with mammals (very small aortic size with limited smooth muscle cell investment), gene redundancy (Zebrafish possess two copies of many mammalian genes), and short of clinical relevance, limit its effectiveness and applicability in modeling human diseases.

On the other hand, mouse models of CVDs described in this project are highly relevant to the clinical setting for patients with CVDs. For instance, patients requiring distal arterial bypass or coronary artery bypass, but who lack adequate autologous vessels for their surgeries, and thus creating a tissue- engineered vessel using stem cells would be helpful. Additionally, by identifying the cellular and molecular targets of CVDs using mouse models, one would expect that new drugs will be developed in the future. Therefore, mouse models for arteriosclerosis research would be essential and useful.

To mimics the clinical settings of human vascular diseases, majority of the animals used in these protocol will be adult mice, which is the predominant animal life stage used by the researchers in over 90% studies similar to our ones. Additionally, adult mice can be handled easily, produce a similar disease phenotype observed in man, and provide enough materials used for analysis to answer the scientific questions.

Under some specific scenarios, such aortic dissection (an injury to the innermost layer of the aorta allows blood to flow between the layers of the aortic wall, forcing the layers apart), mice at juvenile stage (three to four week old) will be used to produce the vascular pathology similar to human aortic dissection.

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

Our group also have excellent work experience on these models. The protocol has been designed to minimise animal suffering by using suitable pain killers during operation. Particularly, the animals will be humanely killed before tissues are removed for experiments or if the animals fail to suitably recover after surgery. It is expected that the degree of distress/suffering will be between mild and moderate for most of the treatments, except that the mice subjected to the higher concentration (>1000ng/kg/min) of angiotension II (a nature hormone in the body that can increase blood pressure) infusion, or received beta-aminopropionitrile (an irreversible inhibitor of lysyl oxidase, an enzyme involved in collagen cross-linking within blood vessels) for 4 weeks, which will lead to sudden death for some animals due to aortic rupture (artery broken). All the operations will be performed by well-trained personal licence holders in the group. Furthermore, we will constantly envisage for procedure refinements and replacements by closely following the new refining disease models and procedures reported in the literatures and increasing using non-invasive imaging techniques to monitor the disease progression.

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

Apart closely following the new advances published by NC3Rs (https://www.nc3rs.org.uk/experimental- design-assistant-eda), and Guidance on the operation of the Animals (Scientific Procedures) Act 1986 published by Home office (https://www.gov.uk/guidance/guidance-on-the-operation-of-the-animals- scientific-procedures-act-1986), following published animal practice guidance will be followed to ensure all the experiments are conducted in the most refined way:

The Experimental Design Assistant. PLoS Biol. 2017 Sep 28;15(9):e2003779. doi: 10.1371/journal.pbio.2003779.

Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. Lab Anim. 2018 Feb;52(1_suppl):5-57. doi: 10.1177/0023677217744587.

Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on refinement. British Veterinary Association Animal Welfare Foundation/Fund for the replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare. Lab Anim. 2001 Jan;35(1):1-41. doi: 10.1258/0023677011911345.

Administration of substances to laboratory animals: equipment considerations, vehicle selection, and solute preparation. J Am Assoc Lab Anim Sci. 2011 Sep;50(5):614-27.

Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci. 2011 Sep;50(5):600-13.

Refinement and reduction in the production of genetically modified mice: sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on refinement. Altern Lab Anim. 2004 Jun;32 Suppl 1A:373-5. doi: 10.1177/026119290403201s61.

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

I and all the PIL holders under this PPL will be required to register and subscribe to NC3Rs (https://www.nc3rs.org.uk/experimental-design-assistant-eda), and closely followed-up all the information/events. Any advances in the 3Rs made in the animal procedures which could be used to address the scientific aims/objectives described in this project will be implemented in timely orders if allowed by NVS and/or home office inspectors.

**A Retrospective assessment of Refinement will be due by 16 September 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?

# 12. Image Guided Therapy for Cardiovascular Disease

**Project duration**

5 years 0 months

**Project purpose**

- Basic research

**Key words**

cardiovascular, imaging, regenerative medicine, disease, therapy

| Animal types | Life stages |
|---|---|
| Mice | neonate, juvenile, adult, aged |
| Rats | neonate, juvenile, adult, aged |

## Retrospective assessment

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

**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 have developed and will apply a range of novel biomedical imaging techniques (MRI, ultrasound, nuclear) which will be able to give more accurate information on what causes cardiovascular disease and more accurate measurements ofhow effective novel treatments are.

We will test a range of novel therapies aimed at reducing damage caused by disease and repairing the tissue that was lost. These approaches will include testing of new drugs to reduce damage from stroke, testing stem cells as means to regenerate the heart, and giving gene therapy that can prevent heart failure.

**A retrospective assessment of these aims will be due by 07 November 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Cardiovascular disease is the leading cause of death worldwide, and its prevalence is still increasing. Hence there is a requirement to find new methods to accurately diagnose pathology and develop and evaluate new therapies to treat heart disease and stroke.

We will develop and utilise our novel imaging methods to directly visualise and measure the effects of these treatments, guiding the optimisation of novel therapies which can be rapidly translated to use in the patients that most need treatment.
What outputs do you think you will see at the end of this project? New information:
New imaging methods will tell us more about how the cardiovascular system reacts to damage and give better information on early diagnosis, disease progression and the effects of treatments.

Image guided therapies will help us develop, test and optimise treatments for diseases including myocardial infarction and stroke. Feedback from imaging the locations and concentration of the therapeutic agent will inform on whether it missed the target organ delivery site or was diluted to an insufficient dose. We can then understand why the treatment was ineffective and modify the delivery route and formulation.

Products:

Novel imaging agents which may consist of contrast agents, biomaterials or nanotechnologies.

Patents and Publications:

The novel techniques developed in this proposal are readily patent-able and will be disseminated by invited talks at international conferences and in leading, open access scientific journals as well as in the media.

Clinical Translation:

Imaging tracers for PET/SPECT/CT, MRI and photoacoustics can be translated to the clinic and we will work closely which clinicians to generate products and techniques which can be directly used in patients suffering from cardiovascular disease.

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

Basic science - The novel techniques developed in this proposal will have a direct benefit for hundreds of academic centres (both nationally and internationally) that are working on cardiovascular disease, therapy and translational imaging methods. Due to the multidisciplinary nature of the project the advances in molecular imaging, gene/cell/biomaterial and pharmacological therapy, and imaging methodologies will impact

within their own specific disciplines.

How information will be disseminated: As the target academic beneficiaries cover a wide range of areas the distribution of information will be through the most appropriate channels – presentations and organisation of relevant conferences/workshops, publications in relevant journals. We will also visit labs to help install and validate technologies and applications developed to ensure their appropriate use. Given the rapid speed in which new technologies are developing, these approaches could have impact within months and be utilised within other Centres in the near future.

Clinical translation – The results will be of value to clinicians as the work proposed here addresses the current barriers that are essential for the clinical translation of cell and biomaterial therapies to the clinic. The ability to accurately track stem cell and biomaterial location over time provides crucial information on how effective the therapy will be and how long the therapy will persist. Timescales for new treatments are long owing to the regulatory processes involved, however, given that some of our research involved combination of multiple devices already approved for clinical trial, a more rapid translation into the clinic will be sought.

Patient benefits: Regenerative therapies are novel treatments for a wide range of cardiovascular diseases including heart attack, stroke, genetic disorders and peripheral artery diseases. Imaging techniques developed and utilised here can be incorporated into the ongoing clinical trials of cell therapy to allow treatments to be monitored and optimised for the individual patient's needs. Experimental therapies will take 5 years to provide substantial benefits in patients. However, new imaging approaches can be much more rapidly implemented as they are often non-invasive and can be incorporated into ongoing clinical trials or patient management.

Clinical investment: The global commercial cell therapy industry was estimated to have an annual turnover of £8.7 bn ($11.2 bn) in 2017 and is estimated to grow to £26.6 bn ($34,4 bn) by 2025. The UK regenerative medicine platform is also investing £45M over 8 years ( BBSRC, EPSRC and MRC) and is working in conjunction with the Technology Strategy Board’s Catapult which represents £1bn of private and public sector investment over the next few years with seven centres of which cell therapies is one. The charitable investment in regenerative medicine was approximately 20% of public funding between 2005 and 2009 which reflects the funding for an emerging technology. It is therefore anticipated that charitable funding will increase significantly.

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

Our team has numerous collaborations within our institution and nationally as part of funded Research Centres. This project proposal will further enhance these specialised collaborations to ensure continuity between the old PPL and this project as well as allowing new collaborations to enhance the multidisciplinary nature of this type of research. These collaborations will include the use of machine learning, where data sets derived from our cell imaging would be used to make in silco models of cell behaviour that may predict treatment response.

Work will be presented across a range of relevant scientific meetings, including British Cardiac Society British Society for Cardiovascular Research European Society of Cardiology American Heart Association International Society for Heart Research International Society of Magnetic Resonance in Medicine World Molecular Imaging

Congress European Society for Molecular Imaging

Tissue Engineering and Regenerative Medicine Society

Research will be published in open access journals with data made freely available to the community

We will also visit labs to help install and validate technologies and applications developed to ensure their appropriate use.

**Species and numbers of animals expected to be used**

- Mice: 3000
- Rats: 1500

## Predicted harms

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

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

The mouse is the most appropriate model species for this investigation as they are the lowest animals in the evolutionary tree in which suitable models of cell therapy can be carried out. in some instances, the small size of mouse organs and vessels makes the rat a more practical model for initiating disease and testing herapies. Larger tissue engineered therapies need to be tested on rats as these permit testing of larger constructs that are more representative of those that will b used in humans

Genetically modified mouse or rat strains that are predisposed to heart failure are required to assess the increased risk of ischemic events in these patient populations. These will be utilised at an early stage of disease progression to test sensitivity of imaging methods and as therapeutic intervention at an early stage is more likely to be effective in animals and patients.

in most instances mature animals will be used, but on some occasions juvenile or aged animals will be used if the effects of cardiovascular disease and therapy are anticipated to differ at these life stages.

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

A major objective of this Project is to develop and validate new non-invasive imaging techniques that can evaluate cardiovascular function in live animals and eventually humans. I high proportion of animals on this project will not undergo any major procedures except for light anaesthesia and non- invasive imaging using biomedical imaging methods like MRI and ultrasound.

The other major focus of this Project is to test the efficacy of new treatments for cardiovascular disease. An example of what would typically be done to a mouse enrolled in a therapeutic study is as follows

Imaging under light anaesthesia

Surgery or drug administration to induce disease

Imaging under light anaesthesia to determine severity of disease

Administration of a therapeutic agent

Imaging under light anaesthesia to determine short term benefits form treatment

Imaging under light anaesthesia to determine long term benefits form treatment

Euthanasia of the animal with removal of organs for further analysis of the mechanisms of therapy using lab techniques.

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

The impacts from non-invasive imaging are low as the animal only receives light anaesthesia for approximately an hour, is closely monitored to ensure heart and respiratory rates and temperature are physiological throughout the procedure, is kept warm and provided with oxygen if needed after the procedure and recovers rapidly after imaging.We will ensure that the suffering induced by the procedures on this licence are kept as minimal as possible for achieving the scientific goals of the research

Surgical, drug and genetic models of cardiovascular disease can have greater impacts on the animals and although pain relief is given during these times with lethargy, lack of appetite and weight loss are common in the first weeks. However, behaviour in the majority of animals, despite have significantly reduced cardiovascular function, is relatively normal by 72 hours after the procedure, with normal levels of feeding, grooming and exercise.

**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)?**
Cardiovascular diseases are the leading cause of death and suffering worldwide. The most appropriate models of these debilitating diseases do unfortunately cause suffering to the animals. However, only approximately 20% of animals will undergo severe procedures, and only a fraction of these will actually experience suffering that approaches the designated humane endpoints. Approximately 30% of animals will undergo moderate procedures such as drug infusions and injection of therapies and most of these will experience little suffering. The remaining 50% of animals will undergo mild procedures such as mild, recovery anaesthesia for non-invasive imaging.

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

- Killed

**A retrospective assessment of these predicted harms will be due by 07 November 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

The objective of this study is to develop translational non-invasive imaging methods which can provide information essential for guiding and optimising novel therapies for cardiovascular disease. Therefore, a complete living animal system is required to incorporate the complex physiology of disease and allow imaging methods and therapies to be developed that can be directly translated to human use. Serial in vivo imaging will be performed prior to and after the onset of disease and therapy so that efficacy can be accurately followed non-invasively over time, similar to the care a patient would receive.

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

imaging of phantoms (tubes of liquid matched to the bio physiological properties of a living organism) primary cells and cell lines Lower species, such as zebrafish In silico simulations

**Why were they not suitable?**

Although in vitro imaging of phantoms (tubes of liquid matched to the bio physiological properties of a living organism) will initially be used to test imaging techniques, this does not incorporate the complex physiology of an in vivo study.

Although drug and gene therapies will be tested in primary cells and cell lines, it is also necessary to test therapeutics in animal models, owing to the complex nature of cardiovascular diseases.

Lower species, such as zebrafish, do not have heart structure similar enough to humans to allow development of relevant translational imaging methods

In silico modelling will be used to design therapeutic experiments and simulate image acquisitions thus reducing in vivo experiments. However, these data will not be able to inform on the complex and unknown interactions between treatments and physiology that occur in vivo

**A retrospective assessment of replacement will be due by 07 November 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?**

To conduct a meaningful experiment, a sufficient number of animals need to be included to confirm that therapeutic benefit results directly from a treatment rather than occurring by chance. More accurate and reproducible the models, treatments and imaging methods increase confidence in the data being correct

Our group has 17 years of experience using biomedical imaging to investigate cardiovascular function and therapy in mice and rats. This has provided a wealth of data on the variability associated with the different disease models, imaging methods and therapeutic outcomes. Hence, our extensive knowledge of the accuracy and reproducibility the models, treatments and imaging methods used has allowed us to use informed statistics to calculate how many animals will be needed to yield accurate and useful data.

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

All in vivo studies will abide by the ARRIVE guidelines and are designed with assistance from the NC3Rs Experimental Design Assistant to allow randomisation and provide advice on groupings, analysis and the minimal number of animals needed to yield an accurate and useful result.

Monitoring animals by in vivo imaging makes a major contribution to the reduction in animal numbers as each animal can be used as its own control, allowing paired comparisons which are inherently sequential. Sequential experimental designs in which the same animal is monitored longitudinally over a number of time points increases statistical power, and uses fewer animals to achieve the same statistical power as conventional designs in which cohorts of animals are required for 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?**

Significant pilot data has already been acquired to inform experimental design, When unavailable, pilot studies will be conducted to optimise experiments. Non-invasive imaging allows multiple imaging methods to be applied to the same animal and hence more scientific information to be acquired, reducing the number of animals that will need to be included in a study. For example, tracking duel labelled fluorescent and bioluminescent cells in vivo can be performed simultaneously, enhancing sensitively.

By using ultrasound guidance for injections into the heart and other organs, we can remove the need for a second thoracotomy of invasive procedure in the majority of animals. Ultrasound guided injection is a method that we have optimised and validated over several years and is an important refinement.

The use of ex vivo assays will enable us to exclude drugs, biomaterials and stem cells that display limited therapeutic potential without the need for living animals, thereby reducing the numbers of animals required for the in vivo experiments.

Histology, fibrosis scoring and immunhistochemical analysis will be used to correlate and quantify effects observed or imaging biomarkers as well as stem cell distribution, number and function.

We ensure that any tissues generated from previous experiments are archived and stored appropriately therefore ensuring that unnecessary repetition of experiments is not necessary.

As part of good laboratory practice, a protocol for each experiment including statements of the objective(s); description of the experiment, including experimental treatments, the size of the experiment, and experimental material; and an outline of the method of analysis of the results will be obtained prior to acceptance of experimental protocol by the PPL holder.

**A retrospective assessment of reduction will be due by 07 November 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

Mice and rats are the most suitable model species for these investigations, as organ anatomy, physiology and injury response is similar to that of higher mammals.Specifically, the heart has 4 chambers that operate in a similar fashion to the human heart, with analogous perfusion, ejection and filling rates. The cell death, inflammatory, fibrotic and remodelling response to injury such as myocardial infarction and pressure overload is also similar to humans. Rats and mice are also compatible with the in vivo imaging technologies that will be employed throughout this project. From a neurological point of view, there are differences in the brain structure of rodents and humans, mainly that rodents are lissencephalic species while primates are gyrencephalic. However, the response to ischemic stroke in respect to timing and routes of cell death and response to injury are similar to primates, making them a useful model system for testing experimental regenerative therapies.

The anatomy and inherent regenerative capacity of lower species, such as zebrafish, make them unsuitable for this research, while at present our imaging capabilities are

limited to small animal, making large animal research unfeasible. Immuno-compromised animals may be used in some experiments when necessary (such as allogeneic cell grafting), but this will not form a major component of the Programme

The models of myocardial infarction, pressure overload heart failure, genetic and drug induced heart failure, peripheral vascular disease and stroke described in this licence are routinely used in research and have been well refined to cause minimal suffering and to provide reliable data whilst still providing useful model system to test therapies. We have extensive experience of the surgical models described and will take guidance from collaborators when needed.

Post insult cardiac remodelling is a progressive and complex phenomenon which evolves over several months. Hence it is important that therapies are tested in the early and late phases of disease and followed up over several months. Animals will be regularly checked for signs of distress over this period and experiments terminated if deemed necessary. Therapeutic agents, including stem cells and pharmaceuticals, will be tested with the aim of limiting the size of stroke and infarct lesions

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

Mice are the most appropriate model species for this investigation as they are the lowest animals in the evolutionary tree in which suitable models of human heart disease can be carried out.

The objective of this study is to develop translational non-invasive imaging methods which can provide information essential for guiding and optimising novel therapies for cardiovascular disease. Therefore, a complete living animal system is required to incorporate the complex physiology of disease and allow imaging methods and therapies to be developed that can be directly translated to human use. Lower species, such as zebrafish, do not have heart structure similar enough to humans to allow development of relevant translational imaging methods. Rats are more suitable for some studies as their heart is ten times larger, allowing surgical grafting or injection of biomaterial therapies onto heart or vessels. The larger size of the rat can be beneficial for increasing imaging resolution of biological structures, such as brain regions or vessel walls.

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

Surgical procedures will always be performed with best practice including pre and post surgery medication and monitoring. All disease models will be developed to cause the least harm by using minimally invasive approaches and lowest doses that initiate relevant disease.

Our use of close chest ultrasound guided injections offers a minimally invasive method for delivery of cells and substances to the animals, refining procedures such as open chest surgery for delivery of therapy.

Using imaging to track the retention of cells and biomaterials in vivo refines the currently used technique of serial sacrifice of animals, with histology performed at each time point

When using imaging measurements of small morphological changes in animals, it is often possible to use milder disease than with other assessments. Non-invasive imaging of

animal disease models can provide earlier humane endpoints and rigorous inclusion/exclusion criteria.

For stroke we will follow the IMPROVE guidelines https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5669349/ and will implement similar refinements and controls across the other Protocols.

We will move animals between cages and anaesthesia chambers using hand cupping on tunnels to reduce stress upon the animals.

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

All in vivo studies are designed with assistance from the NC3Rs Experimental Design Assistant and will abide by the ARRIVE guidelines. Myself and my group regularly consult the NC3Rs resources available in conjunction with our institutional guidance, training and meetings.
However, imaging measurements can often be use to refine protocol with milder disease than with other assessments by providing earlier humane endpoints and rigorous inclusion/exclusion criteria. Our imaging Centre has state-of-the art preclinical imaging platforms and by working closely with the institutions biological services team we aim to incorporate imaging mediated refinement where possible.

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

Our institution has a yearly 3Rs , improving welfare and enhancing science" which is attended by all PPLs and my group regularly attends the numerous NC3Rs events that are organised throughout the year. New methods are often presented at these meeting and during the previous implemented many developments that we heard about such as the development of ultrasound guided injections rather than surgical implantation, awake ultrasound for assessment of cardiac function without the need for anaesthesia, and fast MRI – reducing the time needed to acquire accurate data on function..

**A retrospective assessment of refinement will be due by 07 November 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?

# 13. Improving understanding and treatment of severe malaria

**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**

Malaria, Pathogenesis, Severity, Resistance, Tolerance

| 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?**

To better understand the processes causing and protecting from severe malaria and to test potential new treatments

**A Retrospective assessment of these aims will be due by 13 November 2026**

The PPL holder will be required to disclose:

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

**Potential benefits likely to derive from the project, for example how science might**

**be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**
**Why is it important to undertake this work?**

Malaria is a potentially fatal parasitic disease, transmitted by mosquitoes. The World Health Organization reports that there are more than 200 million cases per year of malaria worldwide, resulting in more than 400,000 deaths, two-thirds of which are in children under five years of age. This is despite huge global efforts and investment to control and reduce the burden of malaria. Progress in reducing malaria cases and mortality has plateaued in recent years, and it is estimated that an additional 6 billion dollars per year would be needed to drive cases down again. Furthermore, the COVID-19 pandemic has interrupted malaria control in many countries and it is estimated that malaria mortality will increase by 50% as a direct result, with potentially much greater effects if donor funding for malaria control is impacted and malaria transmission dramatically increases. Although there is currently one licensed vaccine for malaria, it is poorly effective and only reduces the risk of severe malaria in young children by about one-third. Thus severe malaria is a major Global public health problem, and looks set to remain so for the foreseeable future.

In countries with a high burden of malaria, individuals are repeatedly infected with malaria parasites from a very young age. These individuals develop immunity with increasing numbers of infections, and this immunity follows a well described sequence, first protecting from severe malaria, then from uncomplicated symptomatic malaria, and eventually resulting in parasite densities in the blood becoming almost undetectable. This explains why severe malaria is often a disease affecting the very youngest children, uncomplicated malaria is seen in young children, whilst older children and adults infections are often asymptomatic. In contrast, individuals without prior exposure and immunity are susceptible to severe malaria at all ages. Naturally acquired immunity to malaria is lost when exposure is interrupted, through emigration or malaria control, and so one of the most worrying consequences of allowing malaria cases to increase again in countries where control has been relatively successful, is that a larger number of individuals will be susceptible to severe malaria.

There is compelling evidence that antibodies against malaria parasites mediate protection from symptomatic malaria, but the mechanisms accounting for rapid protection from severe malaria are unknown. Similarly, the mechanisms determining the onset, progression and resolution of severe malaria are poorly understood. To date, no adjunctive treatments have been identified which improve the outcome of severe malaria in human clinical trials, the only intervention proven to save lives is the prompt administration of antimalarial drugs, and the mortality rate of severe malaria remains around 10%-15%. There is an unmet need to better understand the mechanisms controlling protection, pathogenesis and recovery in severe malaria, in order to identify adjunctive treatments which can improve patient outcomes. This need is pressing, because it is likely that the burden of severe malaria will increase over the next decade in the wake of direct and indirect effects of the COVID-19 pandemic. Therefore it is extremely important to conduct translational research using appropriate animal models to investigate mechanisms and test potential therapeutic interventions.
**What outputs do you think you will see at the end of this project?**

This work is expected to provide new information about the biological mechanisms and processes which cause severe malaria, protect from severe malaria, and determine recovery from severe malaria. The primary expected benefit from this is the publication of

new scientific knowledge about these processes which will inform the development of new treatments.

This work may reveal new approaches to treat or prevent severe malaria. The expected benefit is the identification of targets for further drug discovery and possibly evidence to support repurposing of existing therapeutics for early phase clinical trials in humans.

This work will generate additional evidence to support an objective approach to experimental investigation of human disease. The expected benefit from this is the publication of new scientific knowledge derived from this approach, and the wider adoption of this approach in the malaria research community and more widely in other diseases.

This work will generate datasets (primarily gene expression data) which will be made openly available to the scientific community

This work will generate a limited biobank of cells and tissues from mice, with and without genetic alterations, which we will use for future work and will share with collaborators.

We do not expect that the work will lead to generation of new materials (such as new antibodies or cell lines).

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

The data produced in this project will be presented at national and international conferences and published in peer-reviewed scientific journals, throughout the course of the project. The new information will change understanding of how severe manifestations of malaria arise and resolve, and how they may be better treated. In all presentations and publications, we will promote approaches to reduce and refine experimental investigation of human disease using mice. We will store tissues from mice throughout the course of the project and will make these available to the groups we collaborate with.

In the medium term the diagnostics and pharmaceutical industry will be interested in disease biomarkers and novel therapeutic targets we identify.

In the longer term the data generated from this study may enable better treatment of severe malaria in humans, and may also have significant impact on understanding and predicting the impact on susceptibility to severe malaria of different strategies to deploy and sustain large-scale malaria control interventions and vaccination. Ultimately this may lead to benefits for individuals at risk of malaria, for malaria endemic countries, where reducing the burden of malaria will bring both health and economic benefits, and for all countries contributing to the funding of malaria control initiatives.

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

Scientific Output:

*New knowledge about malaria pathogenesis.* These data will be useful to malaria researchers and the pharmaceutical industry, and we will publish these findings in open-access scientific publications. We will publish both positive and negative results and seek to make the complete datasets underlying our findings available upon publication. We will share published and unpublished data, and materials, with our collaborators. We will promote awareness of our findings through press releases accompanying publication;

social media and newsletters; and presentation at major international conferences.

*Advances in the 3Rs.* The same communication channels will be used to disseminate advances to the malaria research community. The Principal Investigator will promote the approaches to achieve the 3Rs more broadly for other diseases within their institution.

Wider Societal Output:

The findings of the project will be of direct value to researchers and pharmaceutical companies seeking to prioritise the development of adjunctive treatments for severe malaria. Our results and associated data resources, publicised as above, will accelerate the development of new adjunctive therapies.

Ethical use of animals in research. The use of animals in research is of great public interest. This project will help to demonstrate a commitment to ensuring that animals are only used in experiments with the greatest relevance to human disease, when alternative approaches are not possible. The project aims and outcomes will be presented to the public through accessible channels such as news stories, social media, and public engagement events.

**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.**

Malaria infections in adult mice recapitulate almost all of the features of severe malaria disease in humans. This means that we can investigate the causal effect of processes and test the effect of new treatments which are directly relevant to human disease.

**Typically, what will be done to an animal used in your project?**
Typically animals will be infected with rodent malaria parasites and the course of infection will be assessed over 5 days to several weeks (depending on the parasite strain) by careful monitoring of their health status and taking small serial blood samples to measure the numbers of parasites and other markers in blood. Animals may have genetic modifications or receive substances which modify biological processes in order to assess the effects on the course of infection, and animals may receive curative treatment of their malaria infection before its peak. Some animals will undergo a second malaria infection after recovery from the first. Some animals will be irradiated before their infection so that they can receive a transplant of cells.

A small proportion of mice will undergo standard surgical procedures under general anesthesia necessary for the derivation of genetically altered animal breeding colonies. **These procedures include vasectomy and embryo implantation.**

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

Mice will experience symptoms of malaria similar to the spectrum of symptoms experienced by humans with malaria. Depending on the parasite strain these range from a transient reduction in activity and feeding which lasts a couple of days, to progressive severe illness involving cessation of normal activity, fast breathing, hunching, weight loss, dehydration, incoordination, seizures and coma. In addition to the malaria infection, mice will undergo additional procedures which are expected to cause no more that transient mild distress (which will be alleviated with appropriate local anaesthetic where appropriate) including: daily or alternate-day small volume blood sampling from the tail to monitor the course of infection, and administration of substances by a variety of routes (most commonly by small injections). In all experiments we apply humane endpoints which allow us to terminate experiments at a stage which achieves the scientific objective with the minimum of suffering.

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

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

Overall we expect adult animals will experience: 30% mild
40% moderate

30% severe

**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 November 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?**

Malaria is a parasitic infection which can affect all organs of the body. The way that the whole body responds to this infection, and the way that this response is coordinated between different cell types and organs, determines the range of outcomes of the infection, from self-resolving mild illness to progressively severe and fatal disease. At present it is impossible to study the concerted response of multiple cell types and organ systems to this sort of infection in any way other than in live animals.

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

We have already conducted extensive work in human samples and tissues, in isolated cells, and in animal tissue, to refine the selection of the processes we will investigate in live animals.

**Why were they not suitable?**

The observational data obtained so far provide strong evidence of association but cannot prove the causal role of selected processes in severe malaria, or demonstrate that treatments targeting these processes actually do improve the outcome of severe malaria. This can only be established in a living animal model, which recapitulates the same processes as those associated with human severe malaria.

**A Retrospective assessment of Replacement will be due by 13 November 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

This estimate is based on our best estimate of the most likely (rather than maximum) number of mice that will be used in each protocol to achieve the objectives of this project licence, accounting for continued use from some protocols. The work proposed in this project licence is staged according to funding. The first stage of work, with funding in place, will use approximately 500 mice.. The second stage is dependent on further funding We have estimated that up to 1500 mice may be required in this second stage.

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

We use the data from our previous work, in human samples, in cell culture, and in mice, to estimate the likely size of the effect of any process or treatment in our experiments. We then use a tool designed and recommended by the National Centre for Replacement, Reduction and Refinement (NC3Rs), called the Experimental Design Assistant, to help us to calculate the number of mice we will need in an experiment to detect an effect of the expected size. We take additional steps to try to minimise numbers of mice where possible by designing experiments which use the same control group for two or more treatment groups, or where some mice may receive more than one treatment and a statistical method is used to determine the separate effects of each intervention (a "factorial 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 sometimes use small pilot studies before large experiments, when we need to optimize design of the larger experiment to allow the smallest number of animals to be used. This might include determination of the optimal dose of a treatment to produce the maximum effect, allowing an experiment with fewer animals to be conducted to detect a difference between treated and untreated groups.

We store as many samples as possible from each experiment including blood cells, plasma, tissue, and organs. This usually allows us to use samples from previous experiments to make measurements which will inform the design of new experiments.

We apply our experience of working with rodent malaria models to all experiments, in order to ensure that we minimise all sources of unwanted variability which might increase the number of animals needed to answer any question.

**A Retrospective assessment of Reduction will be due by 13 November 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

The animal models used will be those with greatest relevance to the disease processes in human severe malaria. The relevance of a range of different mouse malaria models has previously been established by comparing the expression of all the genes turned on and off in these mice with all the genes turned on and off in humans with severe malaria. This allows us to select the mouse models with the least suffering whilst still recapitulating the same processes occurring in human disease.

We have refined sampling methods for monitoring the progress of illness in mouse malaria experiments. By introducing new equipment we can now use the same drop of blood from a single tiny needle prick on the tail of a mouse for measurements of multiple analytes. We have demonstrated that this causes minimal and very brief discomfort to mice, if they show any response at all.

We have also identified optimal routes for administration of many substances, in order to minimise procedural distress and suffering.

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

The most reproducible course and manifestations of malaria occur when adult mice are infected with rodent malaria parasites.

Malaria parasites are very restricted in their ability to infect the red cells of different animal species, and mice are widely-recognised to be the least sentient animal species in which malaria disease can easily be studied. Although natural malaria parasite infections do occur in other species of animals, including birds and reptiles, these species are less suitable for laboratory study and the parasites do not reproducibly cause any disease with similarity to human severe malaria.

Mice cannot be anaesthetised during the course of malaria infections because progress of symptoms occurs over the course of days, which is too long to maintain anaesthesia.

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

After reviewing our experience and records from the last 5 years of the progression of clinical features of illness in each of the mouse infection models we intend to use, we have identified easily recognisable and objective physical signs which we will use to guide the frequency of monitoring and humane endpoints in our experiments. We have discussed these in detail with the Named Veterinary Surgeon and Named Animal Care and Welfare Officer, to ensure that our interpretation of the severity of illness experienced by the mice is correct and in keeping with the severity of each protocol, and will enable us to achieve our objectives without any unnecessary suffering. We have also ensured that these features would be easily recognisable to both the research team and any animal technicians assisting with care of the mice. In general terms these clinical features relate to movement and posture of mice, and are for the most part recognisable immediately upon inspection, without the need for physical intervention or examination. Actions to be taken based on these clinical features have been defined for each infection model, based on the known speed of progression of illness in that model, and whether infections are expected to resolve or would progress to death if left untreated.

In our previous work over the last 5 years we have introduced additional tools to help us to establish experimental endpoints without the need to progress to the humane endpoint. An important example of this is the adoption of the Rapid Murine Coma and Behavioral Scale (RMCBS), which is a score system that has been validated for use in experimental cerebral malaria in mice. Although this does require physical assessment of mice, it has been shown that when a certain score is reached, mice will definitely have typical changes of cerebral malaria in their brains, which can be observed under a microscope. This is very important for us, because it means that we can define the onset of experimental cerebral malaria and can end experiments at this point without needing to progress to the humane endpoint.

Another tool we have introduced is the use of point-of-care tests, which can measure the concentration of analytes in blood using the same drop of blood we use to measure parasite levels.

Whenever appropriate we use local anaesthetic and non-distressing handling methods to prevent distress associated with procedures.

**What published best practice guidance will you follow to ensure experiments are**

**conducted in the most refined way?**

We will follow guidance from the NC3Rs and Laboratory Animal Science Association to refine the conduct of our experiments, including:

Avoiding Mortality in Animal Research and Testing

Guiding Principles for Preparing and Undertaking Aseptic Surgery Breeding and Colony Management
Blood Sampling Genetically altered mice Grimace scales
How to Pick Up A Mouse Procedures with Care

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

The principal investigator and team subscribe to the NC3Rs newsletters, attend institutional 3Rs training courses, and the principal investigator participates in other 3Rs activities. In addition we are in frequent contact with the named veterinary surgeons and named animal care and welfare officer, who often provide recommendations about refinements to specific experiments.

**A Retrospective assessment of Refinement will be due by 13 November 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?

# 14. Investigating the Use of Fat Derived Stem Cells to Prevent Leakage of Bowel Joins After Surgery

**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
- 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**

Anastomotic Leak, Stem Cells, Omentum, Alginate Gel

| 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 project’s aim is to harness the healing capabilities of fat derived regenerative cells (which we have identified in lab-based models) and combine this with a rapid setting gel to develop an implant that will be applied around a join between two ends of bowel to promote healing and prevent leakage. This will help us better understand the healing process at the bowel join line following removal of diseased portions of bowel and build on our lab-based characterisation work in humans and preliminary animal study in a pig model.

**A retrospective assessment of these aims will be due by 26 November 2024**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Whenever two ends of bowel are joined together during surgery there is a risk that the joint (anastomosis) may leak due to poor healing. Leakage from the bowel (anastomotic leak) can occur in up to one in eight cases, and is the most feared complication of bowel surgery. The patient becomes unwell and often requires further surgery with the formation of a stoma, which is often permanent.
Around one in five patients die because of an anastomotic leak. In patients that survive a leak, there can be long term consequences that impact on quality of life. New treatment strategies are required if we are going to reduce the risk of this serious complication.

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

This research has the potential to bring about a step change in clinical management, reducing the risk of bowel join leak and its consequences for patients undergoing bowel surgery. Success with this model will provide early evidence and help to progress the regenerative cell and gel technology towards first-in-man clinical studies. Regenerative medicine scientists will be targeted through open access publication in high impact scientific journals and presentations at national/international scientific conferences. As the research involves mouse models, it will be of general interest to those working in animal research. We will ensure that the animal work complies with the principles of the 3Rs (replacement, reduction and refinement) and the ARRIVE guidelines provided by the Home Office.

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

Short Term (1-2 Years): The methodologies developed will be of use to researchers in the field of regenerative medicine, providing an alternative source of regenerative cells with optimised protocols for harvesting and application. This will be the first time that ODRC/gel implants have been developed and their efficacy tested in animal models of wound healing.

Long Term (3-5 years): This will open up many other avenues for the use of the technology as a promotor of wound healing at other sites of the body.

A) Patients: Up to a third of patients who suffer an anastomotic leak will die. Patients that do recover from an anastomotic leak experience long-term consequences in terms of increased cancer recurrence, high permanent stoma rates, and poor quality of life. If this research is successful, it is hoped that our new intervention will reduce the huge morbidity and mortality associated with anastomotic leak.

B) Clinicians: Many attempts have been made to reduce the incidence and impact of anastomotic leak through advances in surgical technique and perioperative care. Although some strategies have shown promise pre-clinically, they have failed to demonstrate efficacy when translated to clinical practice. As a consequence, the incidence of anastomotic leak remains stubbornly the same as it was 50 years ago. It is hoped that this research will ultimately provide clinicians with a means to reduce anastomotic leak and improve patient outcomes.

C) Healthcare Providers & Policy Makers: In the UK, approximately 100,000 anastomoses are performed each year for gastrointestinal disease, with leak rates varying between 1%-15% depending on the site of the anastomosis. Assuming an average leak rate of 10%, this equates to around 2,000 avoidable deaths and £250m additional healthcare costs per annum. Fewer anastomotic leaks would see a reduction in length of stay following gastrointestinal surgery and a reduction in re-interventions.

D) Industry Partners / Commercial / Private Sector: Preliminary work characterising human regenerative cells was supported in kind by an industry partner. We will continue to develop this relationship and work with a potential commercialisation partner.

E) Society: General Surgery, which includes gastrointestinal surgery, accounts for the highest activity in the UK, with around 1.3M procedures performed per year surgery. The numbers of operations are expected to increase with the aging population and the rise of certain diseases (e.g. increasing cancer incidence). Inevitably, more gastrointestinal operations will result in a greater absolute number of complications, including anastomotic leak, with an increasing socio-economic impact. Anastomotic leak is one of the most serious and most frequent complications of gastrointestinal surgery. Reducing its incidence will make surgery safer and ensure that more patients are able to return to full postoperative physical function with resumption of normal lives and contributions to society.

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

We will disseminate the findings to relevant surgical forums through presentations to the Association of Coloproctology of Great Britain and Ireland, The American Society of Colon and Rectal Surgeons, and the Society of Academic and Research Surgeons. We will present the findings at relevant scientific meetings, such as TERMIS (Tissue Engineering) and The Gordon Conference of Regenerative Medicine. The group will undertake various outreach activities to ensure that the research, and the role of the funders, is recognised by the general public. To ensure that the relevance of the work is easily understood, we will use modern forms of communication, such as Visual Abstracts. Engagement with public and patients will be central to the design and conduct of the research to ensure that it remains directly relevant to end-users. We will work with the patient and public involvement groups to disseminate the work to its network of stakeholders. The group is part of a national network of academics, clinicians, industry partners, and public and patient members with an interest in facilitating the pull through of novel devices and technology into clinical practice

**Species and numbers of animals expected to be used**

- Mice: 83 Male and 83 Female Black 8 week old C57BL-6 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.**

Our early data supports the idea that ODRCs may promote healing of bowel anastomoses and represent a novel means for preventing bowel join leak. However, to test this a definitive preclinical small animal model is required. This cannot be undertaken in pigs due to the numbers and costs involved and the fact that there is no good pig model for anastomotic leak due to the high healing rate. We therefore propose to develop the ODRC/ gel technology and test its safety and effectiveness in an established mouse model of bowel join leak. A recent international consensus statement regarding animal models for research on bowel joins supports the use of a mouse model as the closest to the human scenario. Three models have been developed in the C57BL-6 mouse. Our preference is the model described by Pommergaard et al. (2004), with a leak rate of ~ 40%. This is high enough to allow a demonstrable change and keep the number of animals used as low as possible.

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

Animals will be put to sleep (anaesthetised) and abdomens opened. The omentum will be removed and taken to the laboratory for further tests and will be incorporated into the gel. The bowel join will be made with the animal asleep and the gel containing cells applied around the join. The whole procedure will last no longer than 1 hour. The mice will receive analgesia and fluid through a vein during the procedure and pain relief after. The animal will be allowed to recovery and monitored closely for 7 days before Schedule 1 sacrifice to excise the bowel join for analysis.

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

Recovery from opening of the abdomen: Pain for 7 days which will be mitigated through regular administration of pain relief.

Abdominal pain caused by large amount of bowel leakage (peritonitis): Pain mitigated through regular administration of pain relief and schedule 1 at the first sign.

Abdominal pain caused by a small amount of bowel leakage (Abscess):Pain for 7 days which will be mitigated through regular administration of pain relief.

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

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

The following severities are expected in the animal model described:

Mild: Group Receiving Bowel Joining + Cells + Gel (60-100%) Group Receiving bowel join +/- gel (60%)

Moderate: Group Receiving Bowel Join + Cells + Gel (0-20%) Group Receiving bowel join +/- gel (20%)

Severe: Group Receiving Bowel Join + Cells + Gel (0-20%) Group Receiving bowel join +/- gel (20%)

Non-recovery : Group Receiving Bowel Join + Cells + Gel (0%) Group Receiving bowel join +/- gel (0%)

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

- Killed

**A retrospective assessment of these predicted harms will be due by 26 November 2024**

The PPL holder will be required to disclose:

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

## Replacement

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

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

It is only possible to test the safety and efficacy of our ODRC/gel implant in a living biological model. We have previously undertaken simulated experiments using wound healing models in the laboratory and the results suggest that the ODRC/gel implant increases the rate of wound healing. These results now need to be verified in a biological model.

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

We have already tested our ODRC/gel implant in laboratory models of wound healing including wound scratch assays which demonstrated positive results. Following a small pig study (n=4) the next step is to trial the safety and efficacy of the implant in a larger animal study.

**Why were they not suitable?**

Verification experiments in a living biological model are now required to demonstrate preclinical safety and efficacy prior to first in man clinical trials.
**A retrospective assessment of replacement will be due by 26 November 2024**

The PPL holder will be required to disclose:

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

# Reduction

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

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

Model Establishment: 2 Male and 2 Female Black C57BL-6 mice

Main Study: 81 Male and 81 Female Black C57BL-6 mice. Total = 162 Mice

Assuming a 20% attrition Rate and including model establishment, maximum number = 198

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

We utilised the NC3Rs experimental design assistant to guide the numbers needed to demonstrate safety and effectiveness of the cell / gel technology in a mouse model of bowel join leakage. The study has been designed online with the up to date ARRIVE guidelines and adhering to the principles of the 3Rs. The ideal study design to evaluate effectiveness is a randomised controlled trial where subjects are randomised to receive one of two treatment options.

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

The model of bowel leakage will be performed on 10 synthetic tube models the same diameter as mouse bowel. This will also be completed on 10 schedule 1 mice. A 4 mouse pilot study will be used to establish the model of anastomotic leak. Tissues will be processed such that analysis can be made in replicate within each mouse. We will also perform an interim analysis after 24 animals to verify the animal model of AL and check the sample size assumptions allowing for adjustment if necessary. The ideal study design to evaluate efficacy is a randomised controlled trial. In line with the 3Rs of animal research the control group will contain two sub groups, gel alone and no treatment, to keep number of mice to a minimum. Randomisation & Blinding: Mice will be randomised using block randomisation through SealedEnvelopeTM at a 1:1 allocation ratio. All mice and tissue samples will be labelled such that researchers assessing the effects of the treatment and analysing the results will be blinded.
Control: It is not anticipated that there will be any difference in leak rate between the animals treated in the combined control group. The purpose of including gel alone in the control group is to allow assessment of its integrity analysis. An exploratory subgroup analysis will be performed to ensure no difference exists between these groups.

**A retrospective assessment of reduction will be due by 26 November 2024**

The PPL holder will be required to disclose:

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

## Refinement

**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.**

Using the model described by Pommergaard et al. in 2014 we expect 40% of animals in the control groups (no ODRC/gel) to experience bowel leak. Of those that leak we expect half to experience abdominal pain due to a large anastomotic leak (faecal peritonitis) and undergo schedule 1 within the first 24 hours and the other half to become unwell and undergo schedule one between day 4 and 7 post op. It is believed this model provides the least pain, suffering, distress and lasting harm to the fewest number of animals in order to demonstrate a minimum level of efficacy to move on to first in man clinical trials.

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

The anatomy of less sentient animals does not allow for the formation of a bowel anastomosis. If the mice were terminally anaesthetised we would not be able to measure the healing effects of the intervention at the required post-operative stage.

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

Animals will be reviewed regularly by the research team and staff from the Animal House with the aforementioned pain relief administered to ensure animals are in as little pain as possible. At the first sign of bowel leakage the animals will undergo schedule 1 killing as soon as possible.

**What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?**
NC3Rs, ARRIVE and local guidelines will be followed. In addition the LASA guidelines on aseptic technique will be adhered to.

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

We have reviewed the NC3Rs website regularly to ensure that our practice is up to date, for example the recent change to the ARRIVE guideline recommendations. We are also of the local animal house mailing lists which distributes urgent updates which are reviewed on a regular basis.

**A retrospective assessment of refinement will be due by 26 November 2024**

The PPL holder will be required to disclose:

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

# 15. Gut pathogen and microbiota effects on host health

**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**

Microbiome, Infection, Pathogen, Host-microbe interaction, Bacterial metabolites

| 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?**

To determine the role of bacteria found in the human intestine in influencing our health and wellbeing.

**A Retrospective assessment of these aims will be due by 21 December 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

While we understand that short bacterial infections can have bad consequences these usually only last a few days and occur at specific sites in the body, for example bacterial food poisoning. Recent work is highlighting how infections in the gut and disturbances in the make up of the bacteria in our intestine can have long lasting effects on our health. However, these effects are really poorly understood. Bacteria in the intestine produce molecules that are taken up by us from our intestines, spread throughout ours and other animals bodies, and can affect the ability of our cells to make energy. Energy generation is a critical function of our cells so any blocking of this process can have effects on how our body functions. Using animal models has enabled discovery of these special molecules, what they look like and their bacterial source in the intestine. We now also have a better understanding of how they interfere with energy generation and we are now looking to understand any potential role for them in diseases such as type 2 diabetes mellitus and autism spectrum disorders. These diseases are of interest as the bacteria which produce the molecules are found at higher levels in the intestines of people with these diseases and these diseases are known or suspected to have issues with cells producing energy.

Continuation of this work will now enable us to focus on other diseases where a role for intestinal bacteria is suspected in causing or influencing the disease. These include Crohn's disease and cancer.

Additionally, we will continue our work investigating intestinal infection by bacteria, including repurposing *Salmonella* as a cancer therapeutic. This work has already identified critical cells for inhibiting tumour growth. Continuation of this work offers real promise in helping understand how our immune systems respond to tumours and how infection can be used to guide the immune system to see tumours more effectively.

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

We envisage that we will generate substantial amounts of new information that we will be able to publish in high quality journals. We will also generate substantial banks of tissue that we can maintain for future experimentation ensuring a reduction in animals needed for future research.

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

In the short term the scientific community will benefit from a greater understanding of disease mechanisms in relation to bacteria that live in our intestine. In the longer term we believe that the findings from this work will have broader implications for our understanding and treatment of diseases such as Crohn's disease but also for diseases where it is suspected that bacteria in the intestine exert a to-date unidentified influence. The results of our work we hope will lead to an easier way to diagnose illness or better approaches to combatting disease by developing new diets or probiotics to prevent colonisation by the bacteria that may have a negative influence on human health.

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

Publications will be the main means of dissemination, but we also engage with the public through the media and through production of educational resources such as podcasts and animations as we have done in the past. Conference presentations and invited seminars will be used to showcase and discuss the results with peers.

The *Biorxiv* has proved an excellent tool for rapid dissemination results from all projects and we will continue to use it as a tool for dissemination of both positive and negative data.

**Species and numbers of animals expected to be used**

- Mice: 1800

## Predicted harms

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

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

For our work the use of live animals is essential due to the complex nature of bacterial-human interaction. While models we use in the lab using cells alone have provided us with excellent published preliminary data for this work they cannot replicate the complex interactions between the bacteria and the immune system.

For our tumour biology investigations, laboratory tumour models are insufficient to mimic the interplay between the immune system, bacteria and the tumour. In particular our preliminary work using a 3D tumour model system failed to model the important role that bacteria travelling to the tumour has on attracting immune cells to the site of the tumour and how this can affect the make up of the tumour.
Using a mouse model allowed us to understand in detail what is happening at the tumour site as we could study what the bacteria, the immune system and the tumour were all doing at the same time. This was especially important in helping us to understand why in some cases the tumour stopped growing. We eventually uncovered that the tumour was responding to immune cells stimulated directly by the bacteria, something so complex that we could not have modelled this simply using cells in the lab.

Mice used for infection work will be adult as younger or older mice are more susceptible to these types of bacterial infections making them more difficult and unpredictable to work with. For tumour studies for similar reasons adult mice will be used, and also due to the need to have tumours of a certain size on the back flank, younger mice in particular would not be suitable. For work studying the influence of intestinal bacteria on our health, adult mice will primarily be used but as these molecules from intestinal bacteria are likely found across the lifespan in mice we may in future amend this license and also study their relevant physiological effects on younger and older animals.

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

Mice will typically be pre-treated with antibiotics before having bacteria administered. When bacterial molecules alone are administered, antibiotics will not be necessary. Infection/treatment will be allowed to proceed and monitored regularly to ensure animal

health. At a defined endpoint animals will be culled. For tumour immunology work, cancer cells will be implanted under the skin and allowed to grow to produce a tumour of defined size over a period of approximately 7 days. Bacteria will be administered intravenously to stimulate the immune system at the tumour site. After a defined period, or when the tumour reaches an agreed maximum size, the animals will be culled.

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

Upon infection all animals experience some weight loss as they can lose their appetite. This normally recovers after 24-48 hours. In the case of infection with *Salmonella* Typhimurium diarrhoea may occur later in the infection process, typically 48- 72 hours (the endpoint). Discomfort will occur with tumour growth in the back flank.

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

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

The expected severities range between mild and severe. For breeding of animals severity is mild while in cases of infection with *Salmonella* is severe as a wild type strain of *Salmonella* Typhimurium is used. For all other protocols, either using attenuated *Salmonella* Typhimurium for tumour targeting, infection with *Escherichia coli*, infection with *Lachnospiraceae*, or treatment with the specified bacterial metabolites derived from *Lachnospiraceae*, severity is moderate.

**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 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?**

For our work the use of live animals is essential due to the complex nature of bacterial interactions with their hosts (e.g. humans). While our models in the laboratory using cells on their own have provided us with excellent preliminary data they cannot replicate the complex interplay between multiple types of host cells and bacteria simultaneously. While we will continue to use a number of model systems in the laboratory these only allow a limited interpretation of the infection process with *Salmonella* and *E. coli* species and also

limit our understanding of the widespread influence of bacteria from our intestine on us.

For our tumour biology investigations, tumour models in the laboratory are insufficient to mimic the interplay between the immune system, bacteria and the tumour. In particular our preliminary work using such a 3D tumour model system failed to model the important influence of bacteria on immune cells that resulted in tumour growth stopping. This was essential to our findings about the role of bacteria in delaying or stopping tumour growth.

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

We extensively use modelling involving a variety of cell types in the laboratory and have done so for each protocol outlined in this license. Each has been very informative and has led to, or will lead to, the proposed work outlined here.

**Why were they not suitable?**

The main problem with our *in vitro (cell)* models is their inability to model complex interactions as the research questions we wish to answer with this license often involve complex interplay (e.g. tumour cells/immune cells/bacteria) or effects that are seen at several sites (e.g. our bacterial metabolites affecting brain and liver). Therefore *in vivo (Animal)* modelling has become essential so that we can understand some of the more complex questions going forward.

**A Retrospective assessment of Replacement will be due by 21 December 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

The numbers are estimated to ensure that we would have sufficient animals to carry out each protocol (except breeding and maintenance) enough times to generate data that could address our overall hypotheses regarding the role of these bacteria, pathogens or bacterial metabolites in animal health and disease.

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

We have identified advanced imaging technologies enable the detection of tiny changes in cells or molecules relevant to the infection.
Application of these technologies in our studies will ensure that we do not need to wait for clinical symptoms in our studies of bacteria in the intestine for example. Our intention is to

attempt to utilize these technologies more widely in an attempt to apply them and make similar gains in our other objectives (i.e. identify molecules that indicate disease is going to start, but before it actually starts). This will enable the reduction and refinement of our protocols further.

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

For our work on intestinal bacteria we will use pilot studies to allow us to determine the most appropriate dose of our molecules from intestinal bacteria to minimise the number of animals required for our larger studies. For all our studies we share tissue where possible and harvest as much tissue as possible in each study to create a bank of tissue that we can use in future. This prevents us having to repeat experiments unnecessarily.

**A Retrospective assessment of Reduction will be due by 21 December 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

We will use a number of animal models each of which best replicate the disease we are interested in. In the case of the *Salmonella* Typhimurium infection model for example, the model is designed to allow infection to proceed to a point where inflammation in the intestine, that mirrors that seen in human disease, has occurred for the shortest time period allowing its study before culling the animal to minimise any further suffering.

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

To reproduce disease that is often complex and involves many human cells, as well as having intestinal bacteria that are similar to that of a human intestine, a higher animal is needed. Animal models have been established for these bacterial infections in mice. While other models such as infecting the waxmoth *Galleria mellonella* have been used in our studies previously, they cannot represent the type and complexity of interactions that that occur between bacteria and human cells that we need to study.

Younger animals mount very different immune responses to healthy adult mice and are susceptible to far lower doses of *Salmonella* resulting in a more acute disease of shorter duration. This makes it difficult to use as a model from human intestinal disease.

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

Further refinements are expected as this work progresses as we share ideas with other research groups carrying our similar infection studies. Extensive training will be provided to those carrying out this work on a day-to-day basis to ensure a minimum level of stress for the animals and to ensure all staff are able to recognize the clinical signs of these mouse models. In addition the animals will be housed in conditions that allow specific behaviours such the formation of a microenvironment by providing material so as nesting can occur. The animals will also be provided with easy access to food and water.

Mice will be monitored regularly and scored according to recognised scoring systems. For injections volumes used will be in line with published best practice such as Workman et al. and LASA guidelines and doses will be the minimum required. We will refer to the NC3Rs website for standard and best practice. Doses will be no more than the maximum tolerated dose.

Administration of molecules, bacteria or cells will be carried out by experienced staff using aseptic techniques, using different sites for repeat injections where possible, and using good restraint, slow delivery and small volumes using fine gauge needles. Where possible substances will be given by the least invasive route. Lower doses of bacteria can be administered if adverse effects are found to be prevalent.

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

We will use guidelines such as those published by the Laboratory Science Association (LASA) and follow best practice as described by e.g. Workman et al. (DOI: 10.1038/sj.bjc.6605642)

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

I receive regular e-mails about 3Rs advances. Where relevant advances are highlighted these will be implemented. We will also have regular meetings with the NVS upon undertaking any new work and particularly when it comes to designing pilot experiments.

**A Retrospective assessment of Refinement will be due by 21 December 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?

# 16. Preclinical models of cancer and metastases

**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
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

**Key words**

cancer, metastases, immunotherapy

| 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?**

Our overarching aim is to identify and validate new molecular and cellular mechanisms, therapeutic targets and therapies in cancer, especially for cancer that has spread from the site of its origin to other organs (metastases). This will provide a better understanding of the disease and enable a development of new therapeutic approaches.

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

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

While the treatment options for many primary tumours are improving, cancer becomes in most cases untreatable once it has spread to other organs (metastases). Cancer spread commonly occurs to several organs at the same time, requiring therapies that can target cancer lesions at multiple sites. Our studies are therefore focusing on cancer in difficult-to-treat organs and multi-organ metastases. It is important to undertake this work in order to improve our knowledge and enable the development of improved therapies for these patients.

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

We will generate new information that is expected to result in publications in scientific journals. This will include (i) information on how therapies that enhance the ability of our immune cells to kill cancer work in organs to which cancer typically spreads. We will use this information to develop new approaches that can further increase killing of cancer cells by the immune system. (ii) information on what is needed by cancer cells to enable their growth in different organs. We plan to test whether manipulation of molecules that were shown to be important for cancer growth through our previous studies, can be used to inhibit/kill cancer cells.

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

In short-term, the generated knowledge will provide novel understanding of cancer cell growth and its interactions with the immune cells in metastases, and thereby profit a wider scientific community by informing their work. We expect that in medium term (next 3-5 years) this knowledge will inform the development of improved therapies at the preclinical level. Moreover, there is a potential for numerous existing drugs targeting components of the immune system to be repurposed as cancer therapeutics, based on the information generated through our studies. In the long-term, this is expected to benefit cancer patients by providing improved treatments and hopefully result in an improved survival and a better quality of life. This is further expected to benefit biotech industry owning such drugs, by expanding the market opportunities and generating additional profit. We plan to approach specific companies directly to discuss such opportunities (next 5-7 years).

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

The outputs from this work will be maximized by effectively disseminating new information through talks and other forms of presentations at scientific meetings, internal and external seminars, educational talks and lectures, through collaborations with scientists and clinicians, and through open- access publications. We are part of an extensive collaborative network of clinicians, pathologists, and scientists sharing tissue resources and information.

Disease models developed and refined in our laboratory are being shared with other research groups through direct collaborations.

We have been previously including statements about negative results in our publications that contain positive results, and will continue to do so whenever appropriate.

The outputs are also being maximized through engagement with the private sector with interest to commercialize our approach, which is expected to enable the development of our technology into a product and its translation into the clinic.

**Species and numbers of animals expected to be used**

- Mice: 3600

## 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 mouse models of cancer and metastases because they mimic human cancer, and it has been repeatedly demonstrated that findings from these models and therapies initially developed in mouse models can be applied to the human situation. Thus, studies of cancer and therapies in these models is expected to enable clinical translation of our findings, which is our ultimate goal. We are using adult mice, because we are studying types of cancer that develop in adults. We are also using genetically modified animals of the following types:

Mice that lack a specific cell type to enable us to study its function. This includes immunocompromised mice that allow for engraftment of human cancer and immune cells.

Mice that lack or over-express a specific molecule to enable us to study its function.

Mice expressing specific receptors on T cells (a subpopulation of immune cells) that allow for studies on immune responses.

Reporter strains that express molecules that can be easily detected and therefore allow for tracing of specific cell types or molecules.

Mice with modifications that result in spontaneous cancer development in order to mimic the disease.

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

Development of cancer in mice in order to mimic tumour growth in patients will be achieved by one of the following methods:

Protocol 1 and 2: development of metastases will be achieved by injecting cancer cells into the blood stream via different routes to mimic dissemination of cancer cells to target organs from the blood, as seen in patients; or a mouse will undergo a surgical procedure under anaesthesia to implant cancer cells into the organ in which metastases typically

develop.

Protocol 3: a mouse will undergo a simple surgical procedure under anaesthesia to implant cancer cells into the organ in which a tumour initially develops in humans (the mammary fat pad to mimic breast cancer) or cancer cells will be simply injected into or under the skin (to mimic skin cancer). Cancer cells then disseminate from this primary tumour to other organs, as seen in patients.

Protocol 4: we will use genetically modified animals that develop tumours spontaneously.

Some mice (~20 %) will undergo irradiation to ablate the existing white blood cells in the bone marrow and the modified progeny of white blood cells will be subsequently injected via the tail vein to reconstitute bone marrow with the new cells (Protocol 5). After this, the mice will be transferred onto one of the Protocols 1-4.

Following tumour development, mice will receive a therapy through injection under the skin or into the intraperitoneal space. To determine whether the therapy can inhibit tumour growth, tumour size will be measured 3-4 times during the experiment, either by a direct measurement of the tumour diameter if the tumour is visible (for example skin tumour) or by imaging that enables visualization of inaccessible tumours (for example in the brain or in the lungs). For the latter, the mice will receive an under-the-skin injection of an agent that enables tumour visualization and will be subsequently anaesthetized to perform imaging.

Tumour sizes in mice receiving therapy will be compared to the control group without therapy to determine whether the therapy works. A blood sample may be taken from the tail vein. A typical duration of such experiment is 2 weeks. At the end of experiment the animals are culled under anaesthesia and organs are isolated for analysis.

Genetically modified animals required for the study will be bred under Protocol 6. To this end, mice will be grouped as required for mating.

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

Animals may experience slight discomfort from anaesthesia and surgical procedure for cancer cell implantation, with the vast majority of mice recovering rapidly, showing no symptoms or mild symptoms that do not persist longer than one hour. In a small proportion of mice (<3%) mild symptoms may continue for up to 48 hours. A small proportion of mice undergoing administration of cancer cells into the blood stream may experience stroke after the surgery (~0.3% of all mice) and these mice are immediately culled humanely.

Administration of therapies, white blood cells and blood draw is associated with only a transient discomfort from needle sting. Irradiation has no effect on the animals.

The majority of mice display normal behaviour for the duration of the experiment. However, development of metastatic cancer lesions in different organs in mice under the Protocols 1-4 may lead to specific symptoms once the tumours become larger. At the experimental endpoint, the majority of mice experience no symptoms or mild symptoms characterized by slight under-grooming. A proportion of mice (<10%) is expected to display moderate symptoms including clearly detectable under-grooming, reduced activity and hunched posture, with the duration of symptoms usually not exceeding 12 hours. Only

a minor percentage of mice (<2.5%) may display severe symptoms, in which case a mouse is immediately culled humanely.

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

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

Protocols 1, 3, and 4: moderate category; all mice are expected to experience moderate severity

Protocol 2: severe category; <5% of the mice are expected to fall into this category and the remaining mice (>95%) are expected to experience moderate severity

Protocols 5 and 6: moderate category; <10% of animals will fall into this category and the remaining mice (>90%) are expected 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 03 December 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?**

Use of animals is required to achieve the aims of this project because a whole organism is needed to study systemic processes that involve multiple organs, such as trafficking of immune cells and their progenitors between the lymph nodes, the blood and the tumour. White blood cells mature in the bone marrow, and travel from there via blood vessels to tumours and metastases. This complex process requires a whole organism and can therefore only be recapitulated in animal models. Immunotherapies work only when all these components are present, and can therefore only be studied in vivo.

Cancer cells growing within their natural organ environment strongly differ from cancer cells growing in the cell culture. Sole analysis of cells grown in cell culture is therefore unlikely to identify good therapeutic targets. Complex compositions of different organs cannot currently be recapitulated ex vivo, and therefore these studies at least in part require use of animal models.

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

We are periodically searching the literature for replacement possibilities for our in vivo models using all available resources. Up to date there are no alternatives that could completely replace in vivo models needed for our studies in order to achieve our objectives. However, certain components can be replaced by non-animal alternatives. Non-animal alternatives that we are currently using include an in vitro migration assay, which mimics certain steps of immune cell migration observed in vivo and can be partially used to replace in vivo studies. We also established a protocol for culturing slices of human metastases tissue ex vivo, which can replace the initial studies looking at whether drugs can inhibit cancer growth, which would be otherwise performed in animal models. Notably, cells derived from human metastases from some organs don't readily grow in culture and robust protocols for their growth could so far not be established. We use in vitro assays to demonstrate that therapeutic molecules we are studying can inhibit cancer growth, and to validate a role of specific molecules in cancer. We plan to use a co-culture of cancer cells and blood vessel cells in the future to mimic interactions between cancer cells and blood vessels. Analysis of publicly available data extracted from comparison of human tissue samples under different conditions can also identify differences between conditions of interest and can omit a need for an in vivo experiment, whenever such data are available. We are also considering a use of organ-on-chip models that simultaneously mimic multiple organs.

**Why were they not suitable?**

There are several non-animal alternatives that are suitable to address our aims and we are including these in our studies, as described above. However, current lack of understanding of the in vivo interactions and systemic processes hamper the development of organ-on-chip models that would sufficiently resemble the in vivo situation to allow meaningful studies of systemic interactions involving multiple organs. It is expected that generation of further knowledge using in vivo models is required to enable the development of such complex in vitro models.

**A retrospective assessment of replacement will be due by 03 December 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

The overall number of animals predicted to be used in this project was estimated based on the use in our previous projects. For each experiment, we use statistical approaches to calculate the minimal number of animals that will allow us to obtain significant results.

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

In order to ensure that a minimal number of required animals is used in each experiment, we perform power calculations using NC3R's Experimental Design Assistant. Whenever feasible, one control group will be used as a control for multiple treatment groups, omitting a need for multiple control groups. We will use male and female mice in experiments whenever possible. Multiple readouts will be used within one experiment, reducing the number of required experiments. Tumour growth will be monitored by non-invasive longitudinal imaging in live mice, omitting a need for multiple time points at different stages of the experiment, thereby reducing the number of required animals. Our techniques for tissue analysis are being refined to allow for detection of multiple parameters in one tissue sample, reducing the number of mice required. Tissues are harvested for multiple projects whenever possible. Harvested tissue from each experiment that is left over is stored and inventoried, so it can be used in the future when new questions arise, omitting a need for additional experiments.

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

The mice will be bred only specifically for planned experiments rather than continuously and the numbers of required breeding pairs will be calculated based on the average litter size for a particular strain. Between the breeding cycles we will only maintain two cages of males and females each. We will share harvested tissues between the project, and maintain an inventory of all left-over stored tissues to enable us to use them for additional analysis whenever new questions arise, thereby reducing a need for additional experiments. We will use in vitro assays to replace in vivo studies whenever possible (see "Replacement").

**A retrospective assessment of reduction will be due by 03 December 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

We will use mouse models of cancer and metastases. Depending on the scientific question, we will use different methods to model primary tumours and metastases, as described in detail under the "Project harms". Each chosen method results in the development of tumours in organs in which cancer that we are primarily focusing on

initially develops in patients, or in organs to which the cancer typically spreads. This is important, because the tumours grow in their typical environment, which influences their characteristics.

Discomfort and distress of animals will be limited to unavoidable procedures required for the conduct of sound research. A priority will be given to a method that causes least distress for the animal while allowing for modelling of a specific cancer type. Surgical procedures will be performed under anaesthesia and pain relief medication will be given prior and after the surgery. Animals will be monitored daily and will be culled humanly if showing adverse effects.

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

Modelling of cancer in adult mice is required because this is the life stage at which the types of cancer that we are studying occur in patients. Characteristics of the immune system and characteristics of organs in which cancer develops differ between life stages, and it is therefore important to use the life stage that adequately mimics the situation in patients.

We are using mice because the immune system and the tumour microenvironment in mice better recapitulate human situation than in less sentient animals such as Zebrafish, and therefore information obtained from studies in mice is expected to be better translatable into human situation. Mouse model is an established host model for studies on cancer progression and immune system, and therefore well characterized. A body of literature supports the correlation of cancer biology and therapeutic responses to immunotherapies between mouse and human.

Studies over a longer period of time are required to allow for the development of different stages of cancer and thereby adequately mimic cancer progression in patients, and such prolonged studies are not possible in terminally anaesthetized mice.

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

All surgeries are performed under general anaesthesia. Analgesics are administered to minimize pain. Injection of cancer cells into the blood stream via some routes may cause stroke immediately after surgery in a small proportion of mice (<0.3%). These animals are immediately killed humanely in order to prevent further suffering. Over the years we were able to refine the surgical procedures and minimize the occurrence of stroke, through measures such as efficient disruption of cell clumps prior to the cell injection, optimization of injected cell numbers, and use of mice strains with a low stroke susceptibility.

Whenever possible, we are replacing this route of delivery with other routes that are less prone to stroke development.

Tumours growing under the skin or in the mammary fat pad are removed or the animals are culled before the tumour exceeds diameter of 1.5 cm. At this size the tumours have minimal effect on the animals. In case of tumours that are not accessible on the surface, monitoring of tumour size by imaging enables us to terminate experiments prior to the occurrence of symptoms caused by cancer growth.

Survival data are often critical to demonstrate clinical translatability of novel therapeutic

targets and agents. To this end, we optimized a monitoring protocol for mice undergoing survival experiments and towards the end of experiment the mice are monitored up to 5 times a day.

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

We are following the PREPARE and ARRIVE guidelines.

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

Our group stays informed though the NC3R website and we are aware of the available 3R online resources. We attend and participate in local events organized under the umbrella of the Animal Welfare and Ethical Review Committee. We also participate in interdisciplinary workshops and seminars that bring together different disciplines, including engineers focusing on the development of non-animal alternatives such as organ-on-chip models. We periodically review and discuss the literature with a focus on advances in modelling of in vivo systems.

**A retrospective assessment of refinement will be due by 03 December 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?

# 17. Repair in neurodegenerative disease and injury

**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**

Neurodegenerative Diseases, Glia, Therapies, Neuroprotection

| Animal types | Life stages |
|---|---|
| Mice | embryo, neonate, juvenile, adult, aged, 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?**

In order to develop novel therapeutics for untreatable neurodegenerative diseases our aim is to improve our understanding of the role of glia in neurodegenerative disease progression and evaluate potential neuroprotective strategies.

**A retrospective assessment of these aims will be due by 11 December 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Neurodegenerative disorders such as Alzheimer's disease (AD), motor neuron disease (MND), frontotemporal dementia (FTD) and progressive multiple sclerosis (MS) represent a major public health threat. In the UK alone over 1 million people suffer with an untreatable neurodegenerative disease.
These are devastating progressive diseases that are uniformly without treatments and often fatal. The absence of any effective therapies reflects to a large extent our poor understanding of the underlying cause(s) of these diseases. In order to develop novel therapies – the ultimate goal of our studies – we must first improve our understanding of the "why" and "how" these diseases start and then spread. This knowledge will enable first development and then testing of targeted potential therapies.

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

Our overall research plan is to use our human clinical research including human - patient stem cell studies in the lab to inform targeted animal studies and in turn findings from animal studies that identify promising therapeutic candidates will be tested in human clinical trials.

Thus the benefits from this project are several and include; advancing scientific knowledge about what might initiate these diseases, how these diseases spread through the central nervous system and whether novel therapies are effective in slowing or even stopping disease.

Collectively all new knowledge gained in these studies will be shared and disseminated with the scientific community, patient led charity groups and the public. This will be done through scientific presentations, published papers and public engagement events.

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

As stated above the short term goals are likely to be increased knowledge and awareness that can be shared with collaborators, patient led charity groups and the wider scientific community. This will potentially open up new avenues of research but also inform and shape ongoing research as well as highlight avenues of research that are not worth pursuing.

In the longer term with our close collaborations with relevant clinics we aim to be able to complete the bench-to-bedside circle of discovery-to-translation and take novel findings from largely human stem cell studies in the lab - also called modelling disease in a "dish" , through animal experiments and return to the clinic. We have already progressed significantly along this route in collaboration with our academic and clinical colleagues where we tested approved medicines as well as novel compounds in a human stem cell platform to identify promising candidates for treatment of people with multiple sclerosis. Top ranked candidates have since been taken through increasingly complex systems - that each model aspects of MS - from slice cultures to fEAE (protocol 3 in our licence) to global EAE in our Biozzi-ABH mouse model (protocol 4 in our licence). The results are

very promising and are being prepared for submission to a peer-reviewed scientific journal.

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

As stated above we have multiple collaborations including with several institutes and investigators in the UK and we have established multiple collaborations within the United Kingdom. Collectively all new knowledge gained in all our studies will be shared and disseminated with the scientific community, patient led charity groups and the public. This will be done through scientific presentations, published papers and public engagement events. We also have extensive experience of public engagement through our leadership roles within relevant centres of excellence and frequently advise / engage / give talks to patient groups and charities.

**Species and numbers of animals expected to be used**

- Mice: 10000

**Predicted harms**

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

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

Primarily rodents are used because of multiple factors including that they are mammals and as such contain the same basic structures and cells as all other mammals allowing us to draw conclusions from rodents to humans, although of course this is not perfect. Rodents are small and have large litters thus making them ideal models for genetic interventions as they can breed quickly and multiple times naturally allowing the creation of mammalian animal models in a reasonable timeframe. Our scientific approach is stepwise and always based on evidence and findings. Specifically, we start from the current state of knowledge learned from collaborations and published observations through wet lab based experiments which involve cell cultures into first simpler animal models before progressing to more complex models that better reflect the complexity of human diseases we are researching. Each question requires different stages of life to be utilised thus for example a therapy for Progressive or Chronic Multiple Sclerosis would require an adult model as well as longterm disease course. This is unavoidable if we are to try and recapitulate some of the key clinical and pathological features of progressive MS. However, as stated above, we would first start in the most simple model and incrementally advance into the more complex model only if the research findings justified this stepwise approach. For example, a potential therapy for progressive MS could initially be tested in a simpler model that may well only require 7 days to understand and prove if our intervention has had a positive impact on the cells responsible for loss of insulation of nerves as found in MS. If this is shown - as we have found in previous studies - we can then test this more definitively in the complex animal model.

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

This depends on the disease and question being asked. For example for a therapy for MS we may initially anaesthetise the mice, expose the skull, drill a single hole through the skull to be able to put a fine glass needle into a specific brain region to inject a chemical to damage the myelin (insulation of the nerves) and at the same time add a therapy to

promote new myelin also called remyelination. Mice would be given post-operative care and analgesia to help their recovery and typically this experiment would run for up to 7-10 days before the animals would be killed and the brain removed and processed. If successful, this therapy might then be progressed into the more complex models which would require mice to have immune mediated wider loss of myelin that more accurately mimics the pattern and extent of myelin loss found in people with MS. This model is called experimental autoimmune encephalomyelitis (EAE). During the disease, mice recover and in these recovery periods, for example day 30 post disease induction they may well then have a general anaesthesia and administration of the therapy to a specific brain or spinal cord regions. Again the mice would be given post-operative care and allowed to recover. This experiment may then run for a further 3 months depending on the impact of the therapy. For example if the therapy is effective, we will expect to see sustained behavioural recovery and pathological evidence of improvement

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

Much like the human diseases we are modelling (such as motor neuron disease, Alzheimer's Disease, fronto-temporal dementia, multiple sclerosis) in animals the impacts of these experiments can lead to physical symptoms such as pain, weight loss, paralysis, bladder dysfunction as well as neurological impacts such as abnormal behaviours including anxiety and memory impacts. As with neurodegenerative diseases in humans, the symptoms start small but progress if unchecked. We do not need to go to those lengths in our animal models as we and others have characterised them in detail and only undertake experiments and their timings as appropriate for the experimental question being tested.

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

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

As explained we have developed a stepwise approach to our scientific work thus starting in cells before moving to simple animal models before moving to more complex models or using multiple interventions in a model. In this way the majority of our work is in cell cultures, and then when we do move to animal models our initial experiments (circa 80%) would be in mild to moderate scenarios before only taking the most successful interventions forward into the more complex and potentially severe models.

**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 11 December 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?**

Due to this complexity of these neurodegenerative diseases it is impossible to accurately model them outside of a complex biological system, i.e. in animals. Only mammals have a complex nervous system and sufficiently developed immune-system to readily compare to humans. We are investigating the central nervous system and as of yet no-one fully understands how the CNS will react to a given situation.

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

We will be using human cell cultures from patients but even our most complex cell cultures such as the human myelinating spheres we have recently developed are not enough in of themselves to be able to fully understand a therapeutic intervention. Even though these spheres contain many of the cells that compromise the CNS (such as astrocytes, oligodendrocytes and neurones) they do not contain all the relevant cells including microglia and immune cells nor do they have a functioning blood supply, nor a blood-brain barrier or other immune cells. It is also unclear if the proportions of cells mirror the proportions, as well as the complexity, of the various basic cells that make up the CNS. Finally cell cultures are kept in artificial conditions with media and gases that do not replicate the natural environment nor the dynamics of a fully formed central nervous system in a mammal.

**Why were they not suitable?**

The 3D myelinating human cultures illustrate the strengths (show myelin wrapping of nerves) but also limitations of this system. Specifically, these cultures do not have a blood supply, immune system and are grown and maintained in a highly artificial system in incubators. Thus they are very important as a research resource and will also limit the number of animal studies needed to more definitively evaluate any promising remyelination therapy.

**A retrospective assessment of replacement will be due by 11 December 2026**

The PPL holder will be required to disclose:

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

# Reduction

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

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

Based on our previous experience and returns to the home office over our previous 2 project licences and 10 years' worth of working with rodents

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

By taking a phased approach using our cell cultures as much as possible we have decreased the numbers of animals that will be needed for experiments to simply ask basic questions or for screening purposes.

**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 maintain our rodent colonies using as few animals as possible to keep them viable but not being wasteful. For example in transgenic colonies often wildtype mice, that do not carry any mutation/genetic insert, are born and in several situations we can take these animals and use them to help maintain our wildtype breeding colonies and thus utilise as many animals and decrease wastefulness that way. Furthermore due to the progressive nature of some of our mice (for example the P301S mutant mouse) they only have a very short window for successful breeding and therefore by closely monitoring and planning all breeding we can generate very controlled numbers of offspring and not breed too many animals which would not be used for scientific research. For experiments we will use the lowest number of animals required to give meaningful, statistically relevant results. Also by utilising as much of the tissue as possible – for example separating the brain into the 2 hemispheres we can sometimes double the amount of material obtained for several different methods of analysis thus decreasing the numbers of animals required. We aim to maximise the use of animals as much as possible with multiple data capture from the same animals. Therefore in a single experiment we can capture behavioural, pathological, genomic as well as protein data on the same animal. This will include collection and 'banking' of tissue from ex-breeder animals as well as collecting central nervous tissue from other animals that can be used to generate some of our disease models. Furthermore by having strong collaborations we can share tissue around the world and therefore decrease the number of animals generated and wasted.

**A retrospective assessment of reduction will be due by 11 December 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 aim to identify and modify disease progression in several animal models that mirror human neurodegenerative diseases including multiple sclerosis (MS), Alzheimer's Disease (AD), and motor neuron disease (MND). These will include the development and maintenance of genetically altered animals, where a gene that is shown to have a role in the human disease is modified in the animal to mimic disease and so allow us to investigate disease progression as well as treatments. We have significant experience with these models and have over time refined our endpoints and strategies to reduce adverse effects wherever possible – for example by discovering early loss of neurones in new areas of the brain that are not responsible for a known disability we can utilise animals before they develop signs of disability such as loss of hindlimb function but still be able to use their brains to investigate neuronal loss. Other methods to create animal models that mimic the human diseases will include the use of compounds, drugs and/or proteins to create localised and known consequences of these diseases such as the loss of the protective myelin sheath around nerve cells (a known cause of MS), or the loss of specific populations of neurones (such as motor neurones in MND or cortical neurones in fronto-temporal dementia).

Also by utilising certain mice that show neuronal loss due to a protein that is seen to occur in many different neurodegenerative diseases (in our case tau, known to be involved in not only Alzheimer’s but also motor neurone disease, frontotemporal dementia and even seen in MS) any benefits we observe could be transferred to a much larger group of diseases and therefore potentially impact many more people suffering from these diseases. We have many years of experience working with these models of neurodegeneration and thus by utilising our expertise we can increase the information and potential benefits we can obtain from an animal whilst in tandem decrease the impacts on individual animals and therefore minimise the welfare costs.

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

To accurately model the complex interactions between neurones, glia, immune-system and vasculature that contribute to neurodegeneration and protection requires in vivo systems. Further the rodent models
- as proposed here- of MS and age related neurodegeneration recapitulate pivotal pathological and behavioural features that underlie relevance to human disease. Furthermore neurodegenerative diseases take decades to develop in humans and are progressive over many years and thus immature or terminally anaesthetised animals would not replicate the human diseases we are trying to impact.

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

We have in place multiple methods of post intervention monitoring including daily weighing and handling to check the mice are mobile and alert. The use of post-operative analgesia and where possible anti-inflammatories. Also by providing stimulation within their home environment such as tunnels and houses or birdseed that allows for more natural behaviours such as burrowing and burying food we aim to increase our animals’ welfare.

**What published best practice guidance will you follow to ensure experiments are**

**conducted in the most refined way?**

There are the NC3R, and ARRIVE guidelines but also through collaborations with multiple colleagues we have many contacts within this field who can not only help and advise us and vice-versa but we can also share tissue and materials with to further decrease the numbers of animals that need to be used for this work.

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

By constant contact with our establishment's biological department and their vets we are kept updated about new training, improved techniques as well as the 3R roadshows that happen to also help keep us informed over the course of this work.

**A retrospective assessment of refinement will be due by 11 December 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?

# 18. Development of novel PET and SPECT imaging biomarkers

**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
- 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**

PET imaging, SPECT imaging, radiotracer, drug development, diagnosis

| Animal types | Life stages |
|---|---|
| Mice | neonate, juvenile, adult, aged |
| Rats | neonate, juvenile, adult, aged |

## Retrospective assessment

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

**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?**

This project aims to discover new tools for scanning patients that will allow doctors to better understand diseases and to better treat their patients. These new tools are based on a highly specialised scanning techniques called positron emission tomography or PET and single photon emission computed tomography or SPECT.
**A Retrospective assessment of these aims will be due by 18 September 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

PET and SPECT imaging techniques allow for the study of different processes happening inside the human body by injecting a small amount of a substance into patients. That substance is called a radiotracer and after injection it travels in the patients' blood stream, reaches the target inside the body and emits a signal that can be detected outside the patients' body using a very sensitive camera.

The discovery of new PET and SPECT radiotracers is worthwhile because there is a clinical need to better understand what causes animal and human diseases. New PET and SPECT radiotracers can also be used to detect and measure the amount of medicine that reaches the targeted area of the body. As a result, PET and SPECT imaging with radiotracers are often used as “imaging biomarkers”, i.e. techniques that can provide a measurable marker of biological processes in a living organism. Given that an intact body environment is complex and minimally accessible, imaging techniques such as PET and SPECT, that do not disturb the organism under investigation, are essential to increase our knowledge of how a given organ or system is affected in disease and how medicines act on it. Additionally, PET and SPECT imaging biomarkers could help governing bodies to issue guidelines on patient care and to decide on the utility of new medicines based on the imaging results. Finally, PET and SPECT imaging allows for detection of changes in the normal body earlier than other methods of diagnosis. Detecting disease early will have direct benefits on patients’ health and life quality. It will also shorten patient’s disability and recovery periods by reducing the need for invasive procedures and long rehabilitation processes. This will result in a reduction of the cost of disease management in the healthcare system and the whole society, because patients can return to their everyday live quicker.

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

At the end of this project, we will have new information on in vivo performance of novel PET and SPECT radiotracers targeting a range of critical body functions during health and disease. For example, we will be investigating a new radiotracer that was designed so it can target inflammation inside the body. Inflammation helps the body to respond to insults and can play an important role in maintaining health. The importance and novelty of our inflammation research has been recognised by a patent application. We envision that other radiotracers currently undergoing development in our group will result in further patent applications over the course of the next 5 years. Furthermore, we will continue publishing our findings in scientific and medical journals. Thus far we have published 18 articles containing our findings from the past 4 years. We anticipate this number will double in the next 5 years due to large grant funding secured in 2019 and 2020, which will enable more research into novel PET and SPECT imaging tools. We will also make our new imaging tools available to the research community, therefore facilitating adoption of the new medical technology developed under this PPL. Throughout this PPL, we will share

our images with the research community so they can be re- used by other scientists, therefore contributing to reduction of animal research duplication.

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

In the short- to mid-term, outcomes from this project will enable researchers to better understand what causes animal and human diseases by using innovative PET and SPECT biomarkers. These biomarkers will also allow researchers to detect and measure the amount of new medicines that reaches the targeted area of the body. Within our ongoing PET and SPECT research programmes, our radiotracer for imaging inflammation is the most advanced in the pipeline and we envision its translation to clinical use in the short-term. In the mid-term, our re-purposed radiotracer for quantification of tissue scarring, 18F-fluoroproline, will provide the research community with unique insight on the mechanisms underlying heart dysfunction in the context of cardiovascular disease. Furthermore, in the mid- to long-term, our novel radiotracer for imaging white matter in the brain (i.e. the insulating fatty layer around neurons) will provide, for the first time, in vivo information about critical brain function. In addition to benefits to the scientific community and clinicians, in the long-term, the PET and SPECT imaging biomarkers developed during this PPL could help governing bodies to issue guidelines on patient care and to decide on the utility of new medicines based on the imaging results.

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

We currently have active collaborations with various research groups in Europe, USA and Asia. We will use these research networks to disseminate new information generated in this PPL, including dissemination of unsuccessful approaches. We also participate in study group discussions from key societies, like the European Society of Molecular Imaging (ESMI) and European Association of Nuclear Medicine (EANM); as well as important national imaging networks like Scottish Imaging Network: A Platform for Scientific Excellence (SINAPSE) and UK PET network. We will also use platforms like the NC3R Gateway to further disseminate our findings within the scientific community. This is in addition to journal publications, conferences proceedings and book chapters covering the latest developments stemming from our research and this PPL.

**Species and numbers of animals expected to be used**

- Mice: 1100
- Rats: 1700

## Predicted harms

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

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

Rats and mice are ideal mammals for development of new PET and SPECT imaging biomarkers. This is because the preclinical small animal PET and SPECT scanners are best suited to image rats and mice over a wider range of ages (resolution of preclinical PET systems is on average 0.7-1.0 mm).

Moreover, the delivery of the radiotracer to the target site by blood circulation is central to PET and SPECT imaging, and rodents have a similar circulatory system to humans. Rats have larger size and blood volume compared with mice and, thus, are better suited for long imaging sessions with complete kinetic analysis and blood collection over the scanning duration. Therefore we envision a large number of rats versus mice will be used in this PPL. Notwithstanding, because mouse models are historically abundant in biomedical research, it is logical to include both species for in vivo PET and SPECT studies designed to develop innovative radiotracers. Finally, rats and mice have a short life cycle, therefore are ideally suited for PET and SPECT imaging biomarker projects designed to identify new radiotracers for imaging cell dysfunction due to natural senescence. For all these reasons, in this project we will use rats and mice aged between 2 weeks and 18 months of age, although the vast majority of our work will be conducted in adult rodents (2-6 months).

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

Typically, approximately 50% of the procedures covered in this PPL will require induction and maintenance of general anaesthesia, cannulation of blood vessels (e.g. tail vein, femoral vein and femoral artery), administration of the radiotracer with or without challenge agent (e.g. pharmacological drug to block or displace radiotracer-target occupancy) and scanning over 1-4 hours. At the end of the scanning session animals may be recovered for longitudinal scanning or killed for tissue collection.
Typically, about 15% of the procedures covered in this PPL will require induction and maintenance of general anaesthesia, cannulation of blood vessels (e.g. tail vein, femoral vein and femoral artery), administration of the radiotracer with or without challenge agent (e.g. pharmacological drug to block or displace radiotracer-target occupancy) and culling of the animals for blood and tissue collection at various time points up to 2 hours post-radiotracer administration. About 15% of the procedures covered in this PPL will typically require induction and maintenance of general anaesthesia, cannulation of blood vessels (e.g. tail vein, femoral vein and femoral artery) for administration of the challenge agent or intra-peritoneal administrations of the challenge agent without general anaesthesia or mini-pump surgery under general anaesthesia followed by awake continual delivery of the challenge agent with vital signs monitoring (e.g. blood pressure measurements via tail plesythmography), and culling of the animals for blood and tissue collection at various time points. The remaining 20% of the procedures covered in this PPL will typically require generation of a model of human disease (e.g. systemic inflammation using LPS, demyelination using cuprizone or regional fibrosis and blood flow impairment via surgical ligation of the coronary artery) followed by administration of radiotracer at different disease time points for scanning over 1-4 hours with or without concomitant challenge agent.

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

Potential adverse effects due to chronic tissue irradiation by repeated administration of a radiotracer and exposure to x-rays (e.g. several imaging sessions over time), pharmacological drugs administration (e.g. changes in animal temperature), repeat anaesthesia (e.g. changes in animal weight) and blood sampling (e.g. reduction of blood volume). These adverse effects are expected to be rare and of transient nature (estimated duration not to exceed 24-48 hours). Measures to minimize the occurrence of these effects will be implemented in all protocols (e.g. use of sophisticated blood sampling equipment to minimize risk of blood loss). Models of disease used in this PPL might lose weight, experience transient pain or display lethargic behaviours. All animals will be routinely and

clinically evaluated for any signs of adverse effects throughout the work protocols. Whenever possible, measures to control and revert the adverse effects will be applied throughout the duration of each protocol (e.g. medicine antidotes, increase of animal temperature via heated mat, supplemental/soft foods to encourage eating).

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

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

Approximately 60% of the mice and rats used in this project will have an expected severity limit of mild and most will be classified under non-recovery sub-threshold severity. About 20% of the mice and rats used in this project will have an expected severity limit of moderate; and 20% may reach severity limit of severe, for example during challenge studies or imaging studies with animal models of human disease.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 18 September 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

The development of new PET and SPECT biomarkers rely on the evaluation of the radiotracer performance in a living organism. Because the living organism environment is complex, various functions are interconnected and contribute to the radiotracer distribution in the target tissues, it is not possible to recreate this with other non-animal alternatives. For example, blood circulation is essential for radiotracer delivery to the target. Additionally, the degradation and elimination of a radiotracer by a living organism is a dynamic and unique process that can’t accurately be measured in other types of studies.

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

In vitro assays using cells and high performance liquid chromatography (HPLC) techniques for pre- screening of lead radiotracer candidates prior to initiation of in vivo animal studies.

**Why were they not suitable?**

The development of novel PET and SPECT imaging biomarkers rely on the in vivo performance of radiotracers. The in vivo environment is complex, various tissues functions are interconnected and contribute to the radiotracer uptake and washout from the target tissues. For example, blood circulation is essential for radiotracer delivery or clearance properties. Moreover, in vivo metabolism of compounds is a dynamic and unique process that can't accurately be modeled by in vitro systems, such as cell assays. Additionally, although significant advances in understanding molecular biology at the cellular level has been attained by using cultured cells or tissue slice preparations, such techniques do not provide information about the intact organ or system under physiological conditions. PET and SPECT imaging has the capacity to do this non-invasively. These aspects impede the study of radiotracer properties outside the in vivo intact environment. For these reasons, the use of intact animals is required. Notwithstanding, in this project, we will seek to use alternatives to animal experimentation as much as possible, namely early in the process of novel biomarker development, such as the use of HPLC and cell assays, in order to reduce the use of animals to the minimum essential to effectively achieve this project goals.

**A Retrospective assessment of Replacement will be due by 18 September 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

Based on number of animals used in the last 5 years under my current PPL and expected increase in number of research projects in the next 5 years.

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

The characteristics of each radiotracer or proposed imaging biomarker will be accessed using a multi- step approach. The radiotracers that do not meet the criteria generally accepted for successful PET or SPECT imaging biomarkers at each stage will not be progressed into the next step. For example, at the stage of radiotracer discovery, if the data from in vitro competition assays or HPLC screening demonstrate the novel radiotracer display sub-optimal properties, namely poor affinity for target, low permeability or high plasma protein binding, then it will not be injected into an animal. During the preclinical research stage, if the radiotracer displays poor uptake and unsuitable kinetics in vivo during the early stage evaluation process, then it will not be progressed into further validation using blockade or displacement studies; nor will its metabolic profile be assessed in animals. This multi-step/milestone process will assure that only promising radiotracers are progressed through the pipeline of in vivo animal studies. Moreover, the

use of alternatives to animal experimentation early in the process of novel biomarker development, such as the use of HPLC and cell assays, will reduce the use of animals to the minimum essential to effectively achieve this project goals. The use of in vivo non-invasive imaging modalities, e.g. PET and SPECT, also substantially reduces the number of animals used (up to 80-90% depending on the study) compared with ex vivo dissection and sampling approaches; as it provides real time, longitudinal and multi-parameter imaging data of the biodistribution and kinetics of the radiotracer.

Given the nature of novel PET and SPECT imaging biomarker development, there is minimal statistical analysis required, as the successful completion of the project objectives is assessed by comparing the radiotracer properties with the criteria for useful PET and SPECT imaging biomarker. This criteria includes: confirmation of radiotracer delivery to target site, high affinity and selectivity of radiotracer to target site, low metabolism in vivo, high target:non-target ratios and kinetics suitable to radionuclide half-life and biological process being imaged. At times, comparisons between groups may include, for example, radioactive concentration in tissue or binding in tissue in naïve animals versus drug treated animals. This could be achieved by using, for example, a two sample t-test. Analysis of time course experiments or multi-group analysis (e.g. kinetic analysis or studies with various blockade or displacement drugs) may be performed using other statistical and mathematical analysis, such as two way ANOVA, area under the curve or intersubject variability. When needed, colleagues with statistical analysis expertise will be consulted for advice on analysis. Typically the number of animals per group ranges between 3 and 6. For example, metabolism studies typically require 6 animals per time point per group (usually between 5 and 8 time points and 2 groups), while in vivo imaging studies often require only 3 to 4 animals per group (usually between 2 and 4 groups). There may be instances where the number of animals per group could be up to 12, for example, when the expression of the target in vivo displays large variability across animals (e.g. radiotracers uptake is dependent on hormonal fluctuations across female animals).

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

The characteristics of each radiotracer or proposed imaging biomarker will be accessed using a multi- step approach. The radiotracers that do not meet the criteria generally accepted for successful PET or SPECT imaging biomarkers at each stage will not be progressed into the next step. For example, at the stage of radiotracer discovery, if the data from in vitro competition assays or HPLC screening demonstrate the novel radiotracer display sub-optimal properties, namely poor affinity for target, low permeability or high plasma protein binding, then it will not be injected into an animal. During the preclinical research stage, if the radiotracer displays poor uptake and unsuitable kinetics in vivo during the early stage evaluation process, then it will not be progressed into further validation using blockade or displacement studies; nor will its metabolic profile be assessed in animals. This multi-step/milestone process will assure that only promising radiotracers are progressed through the pipeline of in vivo animal studies. Moreover, the use of alternatives to animal experimentation early in the process of novel biomarker development, such as the use of HPLC and cell assays, will reduce the use of animals to the minimum essential to effectively achieve this project goals. The use of in vivo non-invasive imaging modalities, e.g. PET and SPECT, also substantially reduces the number of animals used (up to 80-90% depending on the study) compared with ex vivo dissection and sampling approaches; as it provides real time, longitudinal and multi-parameter imaging data of the biodistribution and kinetics of the radiotracer.

**A Retrospective assessment of Reduction will be due by 18 September 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**
We will use naïve rats and mice (c. 70% of all animals used in this PPL) as well as pharmacological or surgical challenges to generate models of inflammation, fibrosis and demyelination (c. 30% of all animals used in this PPL). To minimise animal suffering, the duration of the experiments will be kept at the minimum required to reliably collect data from the experiment. The imaging studies will be conducted under general anaesthesia, and the radiotracers or any substances will be administered via the least invasive route and the smallest possible volume. These methods will minimise animal suffering and distress. Typically, in vivo imaging scanning sessions will not exceed a maximum of 6 hours. The exception to this would be a radiotracer that displays a very slow elimination from the target tissue and would, therefore, require imaging scanning to be acquired with different time intervals. In these cases, in vivo imaging scanning sessions will not exceed 6 hours per session, with a maximum of 12 hours scanning over a 36 hour period. Furthermore, an automatic blood sampler apparatus will be used when collecting various blood samples throughout the imaging period. This instrument represents a refinement of the blood sampling technique, as it substantially reduces the amount of blood necessary to obtain meaningful data. We have also developed a new technique in our group that allows for analysis of drug PK/PD and radiotracer metabolism in sub-millilitre blood samples, therefore further refining the blood sampling procedure while reducing the number of animals required for a given study by allowing for multiple blood collections per animal.

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

The animal species to be used in this project need to be rats and mice. The preclinical PET and SPECT scanners are best suited to image rats and mice. Moreover, the delivery of the radiotracer to the target site by blood circulation is central to PET and SPECT imaging, and rodents have a similar circulatory system to humans. Rats have larger size and blood volume compared with mice and, thus, are better suited for long imaging sessions (up to 6 hours) with complete kinetic analysis and blood collection over the scanning duration. Mice are more amenable to genetic alterations compared with rats, thus providing a good platform for the development of animal models of human diseases, which would be valuable for long term application of the novel radiotracers (developed in this project) in biomedical research. Most animals used in this PPL will be terminally anesthetised (>50% of total animal number).

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

We will increase monitoring and post-operative care of animals undergoing longitudinal imaging studies or PK/PD experiments. Pain management following surgical procedures involving recovery of animals from general anaesthesia will be monitored closely and daily. Animals undergoing procedures without general anaesthesia (e.g. dosing via intraperitoneal injection or blood pressure measurement) will be gently handled and trained, in order to minimise distress.

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

We will follow published PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines when conducting our research. In accordance with 3R principles, we have chosen to use rats and mice for our research because they represent the most tractable model for the in vivo study of radiotracer biodistribution and binding kinetics given the availability of commercial and well- characterised strains. Importantly, the use of rodents is a refinement compared to other higher mammalian organisms as they have a lower degree of neurophysiological sensitivity. Furthermore, rats and mice are an appropriate model since they reproduce many features of the human physiology (e.g. blood circulatory system) relevant to this project.

Our group is committed to ensuring the most refined protocols are used in our studies. To this end, we pioneered the use of an acquisition and reconstruction method to standardise imaging protocols with the NC3R support , as well as the development of new protocols for kinetic modelling experiments using sub-millilitre blood collections. This represents a significant refinement to studies that use non-standard imaging protocols with high radiation doses and large blood volumes for kinetic modelling experiments.

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

Our establishment organises a number of events each year, including the 3Rs day, that aim to raise awareness and train students and staff on best practices to minimise animal suffering, improve welfare of animals in research, and support the development of alternatives to animal models. Myself and my team routinely attend these meetings and will continue attending them throughout the course of this project.

**A Retrospective assessment of Refinement will be due by 18 September 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?

# 19. Identifying and targeting stress pathways in cancer formation and progression

**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**

cancer, autophagy, therapy, homeostasis

| 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?**

To identify stress response pathway genes that control cancer incidence and outcome, and understand the mechanisms underpinning this. Building upon this knowledge, identify drugs that can manipulate these pathways to treat cancer.

**A Retrospective assessment of these aims will be due by 08 July 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

The survival and continued multiplication of cancer cells is strongly affected by "stress response genes" in those cells, either directly or via other cell types in the tumour (such as immune cells that can kill cancer cells).

By understanding and manipulating these genes we could thus eliminate cancer cells in patients, or reduce the incidence of cancer formation. This is particularly important for cancers that have a poor prognosis, including pancreatic and lung cancer.

This work will test the function of novel genes involved in stress response pathways in mouse models of such cancers, as well as in certain other models of cancer that are chosen to allow easy discovery of some of the general principles of stress response pathways (for example, skin (squamous) cell carcinoma models are very good for studying immune responses to cancer). Then, we will find drug molecules targeting these responses. Thus, we will establish the first steps on a journey toward one day the medical application of manipulation of stress response pathways for improved cancer survival.

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

This project will give new information showing that previously unsuspected genes and processes participate in cancer. It will identify new drugs that can exploit these genes and processes for prevention of cancer or treatment of cancer. This will be done particularly with an emphasis on repurposing existing non-toxic compounds that are already known to be safe in human patients. All of this information will also be published for others to build upon to further the quest for better cancer treatments.

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

The knowledge produced will help us understand how cancer forms, develops and is sustained. This includes currently hard-to-treat cancers, such as lung and pancreatic. Short term, this will contribute to our basic knowledge and understanding of cancer, steering investigations of cancer biology down fruitful avenues for further understanding of this disease. Medium term, our investigations will allow identification of new drugs that might be trialled for improvement of cancer outcomes. Understanding the mechanisms that regulate cancer will also allow existing anti-cancer drugs to be better matched to the most appropriate patients in the clinic. Thusly, long-term this work will contribute to improved survival from cancer.

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

Sharing of the knowledge generated by this project will be achieved by promoting our studies at international conferences and through both publication on pre-print publication servers and via publication in high impact scientific and medical journals. All data will be published in one of these formats, including negative data to help avoid unnecessary repetition of animal experimentation by other researchers in future. We will also work with clinical colleagues to help work out how to test new therapies based upon our findings.

**Species and numbers of animals expected to be used**

- Mice: 9900

## 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, to generate cancers in the sexually mature late-stage juveniles and adults. This is because cancers we study (for example, lung and pancreas) commonly affect adults in humans; the models we propose to use have been extensively developed and characterised in adult mice. They provide excellent mimics of these human cancers, in terms of both the cells in the tumour, their organisation in the tumours, and the underlying changes in the DNA and gene activity in the tumour. Mice are the least complex animals that have been able to model all of these aspects of human cancer. These animals can be used to test the effects of genes or drugs on the formation and progression of tumours (modelling the incidence of cancer and the outcome of cancer, respectively). Adding to this, the technology for genetic modification of mice allows precise manipulation of their DNA to address these questions successfully. Indeed, many of the models of cancer that rely on changes in genes that promote cancer have been made in mice and are extremely well-characterised here - this allows "benchmarking" of our results in this particular organism against the results published by other researchers in other arenas of cancer research. In turn, this maximises the scientific benefit of our studies by using this particular organism. Finally, in mice that can be implanted with tumours, very well controlled and informative experiments can be done to obtained maximal insight into the effects of genes and drugs on the survival and growth of cancer cells, and the potential rejection of tumours by the immune system.

Sometimes tiny tumours that have no effect on the health of the animal start to form in late-stage juvenile mice using genetically-modified mice, for example 8-10 weeks old. Similarly, the optimal age for injection of cells to form tumours is around 6-8 weeks old and thus injections are done in late-stage juvenile mice (although, again, tumours are tiny at this point). Mostly, by the time tumours that have potential to cause harms form, mice are adults.

To generate some of the genetically-modified adult mice to study cancer, we will also mate together adult genetically-modified mice to keep the genetically-modified bloodline going and to yield animals for such experiments. Thus, the project also technically uses embryonic, neonatal and juvenile mice, although no cancers develop in these animals; juvenile mice have a small amount of ear material clipped off for identification purposes and the mice are then housed without further procedures being done to them until they become adults.

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

A typical animal undergoes either one of three following:

The animal is ear clipped to mark it for identification, and then either used for mating or kept

to maintain genetic stock without further significant procedures being performed upon them.

The animal is born with genetic alterations that cause cancer. Usually this requires a further injection into the abdominal cavity, or inhalation, of an agent that will trigger pancreatic or lung cancer formation, respectively. After this, animals are carefully monitored for health to avoid missing any symptoms of tumour burden. They may also be anaesthetised upon occasion to enable non-painful monitoring of tumour growth by X-rays or light ("imaging"). Some animals may also be given drugs that are potentially useful anti-cancer drugs, usually by injection or by supplementation of their diet. In most cases animals will be killed prior to obvious harm and suffering being caused by the cancer. Tumour growth size and the composition of tumours can be compared between animals at this time, allowing us to measure the effect of different genes or drugs. Tumour growth is slow and symptomless in most cases. The mice may be kept alive for up to 30 weeks, typically, for tumour growth, with drug treatments and monitoring of growth daily or weekly (depending upon the overall length of the experiment).

The animal undergoes anaesthesia and brief (less than 15 mins) surgical implantation of cancer cells by injection into the pancreas, once the abdomen is partially opened, or under the skin injection of cancer cells to form a tumour. Tumours develop over a period of three to four weeks where the cells were injected. After the surgery route, animals rarely exhibit harmful symptoms of tumour growth. Mice where tumours form in the skin can, however, develop ulcers, which are monitored carefully to ensure healing. As above, animals may also be monitored by imaging and/or be given experimental anti- cancer drugs.

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

The typical outcome of injections or other substances being given to mice is momentary discomfort or pain to the animal. Animals generally recover well from anaesthesia and surgery (with pain relief to ease pain or discomfort). Complications in less than 1% of cases, either toxicity from anaesthesia causing breathing issues, or non-resolvable pain, are dealt with immediately by killing the animal.
Drugs are chosen and given in a way generally not associated with significant side effects.

So, the main adverse effect of this project is tumour formation, although this only produces symptoms in a minority of animals prior to the end of the experiment. The main symptom of tumour burden in either the lung or pancreas is gradual weight loss, which may occur over weeks. If this gets near the point where it would affect the welfare of the animal the animals are killed immediately to prevent unnecessary suffering. Animals may also show signs of general unwellness and distress, potentially involving pain, for example seen as the animal becoming withdrawn and hunching. Animals are checked daily for this and killed immediately if these effects are detected. So, these symptoms are present for hours, not days. Animals with the skin tumours frequently suffer from skin ulceration. This is checked extremely carefully to ensure healing. Animals may have ulcerated (but subsequently healed) tumours for two to three weeks on average.

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

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

Most of mice on this project will be subject to no significant harm as they are used for breeding purposes (70%)

The remainder of mice are used for tumour formation. The majority of these (around 25% of all mice overall) suffer no harm greater than being exposed to anaesthetic, injection of non-toxic drugs, etc. (thus we class these as experiencing "MILD" severity).

Some of the mice developing tumours do experience adverse effects due to cancer symptoms, as outlined above. These mice are humanely killed before the disease can progress. These mice are considered to have experienced "MODERATE" severity outcomes (less than 5% of all mice).

A small minority of mice with skin tumours develop ulcerated tumours and they can suffer, particularly, if these fail to heal, as shown by bleeding for more than one day or "necrosis" (tissue is locally starting to die off around the tumour), limited to the injection site. This failure to heal is considered a "SEVERE" outcome and these mice are humanely killed (around 0.4% of all mice on this project).

**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 08 July 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?**

Tumours are made up of different cell types (including immune cells, blood vessel cells, amongst others). Their growth is controlled by the three-dimensional arrangement of these cells, within a specific organ in the body, which also exchanges fluids and other cell types with the rest of the body. Currently there is no non-animal model that can model this complexity in its entirety.

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

We have considered organoid models, which are three dimensional cultures of cancer cells embedded in a tissue-like structure. These present a limited, but more diverse range of cell types from the tumour, and have been shown to be more accurate in modelling cancer, than traditional flat cultures of cancer cells in a plastic dish. They have replaced some animal experiments in the lab (see below).

**Why were they not suitable?**

Lung organoids are still at an early stage of development and offer limited scope for replacement of mouse models. Pancreatic organoids fail to model the major cell type within the pancreas ("acinar") from which tumours develop, preventing study of tumour initiation and cancer risk. Organoids of pancreatic cancer have been developed, however. These can offer some modelling of gene and drugs controlling tumour growth, and can incorporate some other cell types from the cancer, other than the cancer cells, albeit with difficulty. These do have some use to replace animals (and we have reduced predicted mouse numbers by using these in the lab). However, in many other instances they still fail to reproduce the required complexity of a cancer and cannot replace our animal experiments.

**A Retrospective assessment of Replacement will be due by 08 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

These numbers are estimated upon the simplest experimental design possible that allows powerful scientific insight, using appropriate statistical calculations.

For the tumour models that required breeding of genetically altered mice, these are complex and our best breeding performance to date suggests we need an approximate 4-fold greater number of breeding mice than those used in mouse tumour models. The mice only actually get cancer in the latter group; breeding with no tumour formation will be the experience of the majority of the animals to be used on this project overall (around 70%).

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

For experimental design, experiments are kept as simple as possible on the whole: one control grouping versus a grouping where a gene is deleted or a drug is given at one dose, with a comparison between these two pairs. Sometimes, a four way experiment, for example where a gene is deleted and drug is given at one dose to look at how whether if a gene is active or not predicts drug response of the tumour. These setups minimise the number of animals needed for statistically relevant and scientifically useful data.

Where appropriate, we plan to use imaging by for example light or X-rays to monitor tumour growth over time in animals - this can lead to better quality data on tumour growth. This in turn can make it possible to replace killing mice at different time points to look at how big tumours are, reducing overall numbers of mice needing to be subjected to tumour formation.

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

Based upon our experience we use the most effective breeding strategies to generate mice that will develop tumours, therefore reducing unnecessary breeding of mice.

We are using a lung cancer model that uses a dosing agent (virus) to provide some of the genes required to start tumour formation, rather than breeding different mice containing these genes together. This results in less unnecessary mouse breeding also.

Where possible we also ensure that the genetic alterations in our animals are "conditional". This means that the changes in the genes will only be switched "on" in a small number of cells. When tumour formation can be switched on at a specific time in this way, the variability in tumour size between animals is less, so we need fewer mice to acquire statistically robust data.

Pilot studies are used when we need to optimise doses of drugs, and to gain preliminary information on statistical parameters to enable planning of full-scale robust experiments that use no more mice than absolutely required.
**A Retrospective assessment of Reduction will be due by 08 July 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.**

"Genetically-altered" mouse models of lung and pancreatic cancer have been developed and refined over many years by the research community. These are mice that have various altered genes such that tumours forming in the pancreas or lung at predictable, well-characterised rates. So, while growth of tumours is necessary to allow us to study cancer formation and growth rates, and responses to drugs, our knowledge and experience of these sophisticated models allows us to design analysis strategies based around killing mice at a predictable time, before symptoms of cancer become obvious.

Furthermore, although tumours have historically been studied by injecting tumour cells underneath the skin of animals, rather than letting tumours form "deeper" inside the body, this is now known to be a poor model for pancreatic cancer, which has a very unique host tissue in the pancreas. By surgically implanting pancreatic cancer cells into the pancreas in some of our models, we obtain highly relevant tumours that rarely cause the mice any

adverse symptoms.

Finally, skin tumours, where tumour cells are implanted under the skin in mice with a fully-functional immune system, don't require surgery and can model a variety of tumour types to discover general principles of immune rejection of cancer. These models are therefore chosen for general studies on principles of tumour immune rejection, and testing drugs that aim to enhance this. However, these tumours do ulcerate (usually healing) and have to be extremely carefully monitored to intercept any more harmful consequences after ulceration. They are viable models with this level of care.

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

The cancers being studied in this project are slowly developing, progressive diseases of adults, associated with long term interactions of the cancers with other developed tissues of the animal. There is no means to model this in animals at a more immature life stage. Non-mammalian systems (e.g. zebrafish) have a place in modelling some of the very fundamental aspects of cancer, in broad terms, but, in comparison to mice, are not similar to humans enough in terms of the architecture, and gene and drug responses of the various cancers that we study.

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

In this project, we are now using our cumulative knowledge of many of the models to implement a monitoring strategy based upon weighing animals periodically over time, in addition to checks for visual signs of harm. We have found previously for lung cancer models that mild weight loss acts as a good first indicator of early tumour growth and can act as a "sentinel" symptom to heighten the scrutiny applied by monitoring (for example moving to daily monitoring of weight). By this "belt and braces" approach, we minimise the duration of time any animal shows symptoms of cancer prior to humane killing. Pancreatic cancer models will benefit from the same approach and we propose to implement this in this project. Nevertheless, development of better indicators is a moot point for many animals as we do not deliberately age them until they get sick from cancer, but rather assess tumour growth at defined times *prior* to mainfestation of sickness.

Where possible, we ensure that the genetic alterations in our animals are "conditional". This means that the changes in the genes will only be switched "on" in a small number of cells restricted to the organ of interest. Thus we breed mice with no adverse risk of inappropriate cancer formation, during breeding or in the wrong tissues.

Post-operative care after surgical implantation of pancreatic cancer involves close monitoring, use of pain relief and careful infection control (e.g. antibiotics, but only when necessary).

We will ensure that all animals receive the highest standard of care, with appropriate social, environmental and behavioural enrichment. We use vented cages to avoid infections spreading between animals. Updated best practice will be implemented on an ongoing basis. For example, we have recently switched away from using handling animals by the tail to cupping animals, which is less distressing for them. Furthermore, mice with tumours on the skin will be housed with soft bedding to reduce ulceration from these tumours rubbing upon the bedding. We are also investigating how to reduce the ulceration of subcutaneous models by selecting new variants ("subclones") of tumour cell

populations, which will hopefully have growth rates that still allow useful study of effects of drugs and genes, but without such a high frequency of ulceration. This may yet further reduce the harms consequent from the minority of problematic ulceration events.

**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. Br J Cancer (2010) 102(11):1555-77.

Smith, A. J., Clutton, R. E., Lilley, E., Hansen, K. E. A., & Brattelid, T. (2018). PREPARE: guidelines for planning animal research and testing. Laboratory Animals, 52(2), 135–141. http://doi.org/10.1177/0023677217724823)
NC3Rs experimental design assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda)

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

Our institution runs regular training events on 3Rs and we undergo training for all procedures to be conducted on animals, renewed to latest standards every three years. All individual experiments are ethically reviewed by a named veterinary surgeon, all to ensure compliance with the 3Rs.

**A Retrospective assessment of Refinement will be due by 08 July 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?

# 20. Elucidating brain tumour cell plasticity and preclinical treatment options during malignant progression and tumour recurrence

**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
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

**Key words**

Cancer, Brain tumours, Tumour recurrence, Therapy, Malignant cellular plasticity - cancer stem cells

| 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?**

Better understand why brain tumours progress and recur despite treatment, and developing and testing treatment options.

**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 it's aims and if not, why not?

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

**Why is it important to undertake this work?**

Malignant brain tumours aggressively invade and grow within the healthy brain. Across the world, 250,000 people are diagnosed with brain cancer every year, and this devastating disease is responsible for 189,000 deaths per year, of which 3,500 deaths occur in the UK annually.

Brain cancer is devastating for patients and their loved ones. Even with the best treatment, patients with the most cancerous brain tumours live for only 12-15 months. On average less than 1 in 20 people live more than 5 years, which is a much worse outcome than expected for most other cancer types.
Treatment fails because brain cancer cells are very adaptive and grow finger-like projections into the surrounding healthy brain. Unlike in other cancers (e.g., breast), brain structures infiltrated by the cancer cannot be cut out whole, so cancer cells always remain after surgery. These remaining cells resist being killed by radiation and chemotherapy so new tumours eventually regrow. Better anti-brain tumour treatment options are urgently needed, and the UK government and cancer charities, Cancer Research UK, have both stated that brain cancer urgently needs more research to improve outcomes for affected patients. Our main goal is to ultimately translate our most promising research findings into clinical applications.

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

The herein described combination of brain cancer development, growth and relapse animal models represents a rare resource for the UK; the absence of which has hampered the clinical use of results that were obtained in laboratory (preclinical) research studies. Clinical testing of potential brain cancer medicines often fails to show any overall survival benefit for patients. The reasons for these setbacks are complex, including potential failure to achieve sufficient concentration of the tested medicines in the brain tumour, underestimating the ability of tumour cells to adapt and survive the treatment, and failure to identify and target the optimal genes and molecules in brain cancer cells so that their growth and can be efficiently halted. This 5-year project seeks to model and compare key stages of a brain tumour patients' journey, which can include surgical removal of tumour mass and observation of the remaining disease that inevitably leads to brain cancer relapse. Biological investigation of the brain cancer at different stages is expected to identify targets that would be overlooked in conventional studies of just the tumour mass that can be largely removed by neurosurgery. Furthermore, potential medicines against promising brain cancer targets can be tested so that the results can inform the planning of how to safely use such candidate medicines in humans (a requirement before any clinical trial testing a potential medicine can start).

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

Short term, our project, objectives, and protocols are expected to offer several insights and possibilities for the wider research community, in particular holding key advantages over existing models that do not take brain cancer relapse into account. For example, target identification in tumour cells in the remaining disease after removing the 'bulk' of the tumour (by micro-surgery) will aid the development of new treatments, and validation of previously identified ‘Achilles heels’, for example using agents that inhibit genes and proteins that allow the tumour to grow uncontrollably.

In the longer term, a clinical patient pathway-mimicking brain cancer modelling strategy that tracks tumour growth and also recapitulates the 3-dimensional structure of a tumour cavity after neurosurgery enables cross-disciplinary collaborations and further research involving engineering (e.g. ultra-high precision image detection), biomaterials (e.g. localised drug delivery) and medicinal chemistry (e.g. repurposing of existing drugs that are effective in vitro but do not enter the brain due to the blood-brain- barrier that keeps many potential medicines out of the brain).

The goal is to develop our most promising laboratory findings to a stage where they can be tested in humans. Currently, we are in the process of building on published results working towards developing a (so called) lead compound (synthetic small molecule) from an experimental chemical that is not yet optimised for use in humans. Before use in the clinic, experimental chemicals showing promising preclinical efficacy in animal models need further improvement via the so-called drug development process. On average, it takes a new drug 12 years to get to market (including R&D, preclinical testing, human trials, and EMA/FDA approval). We hope to test improved (drug-like) analogues of the candidate medicines (synthetic small molecules) during this project, which may inform the planning of first-in- human clinical trials. However, even if all research progresses as planned, the impact for patients with regards to clinical trials and new treatment possibilities reaches beyond the lifetime of this project.

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

The project team has been put together to maximise impact across brain cancer research by immediate collaboration and knowledge exchange with other UK-based groups. We have a wide-ranging international research network and have a track record of collaboration with industrial partners.

In addition to the earliest possible academic publication of sound research, for example via making our most timely manuscripts available via a widely used preprint server that features timely research before peer review, members of this project team regularly engage in laboratory tours and science activity days. We regularly give presentations for brain tumour patients and their relatives, fund-raisers, support groups, and charity ball/festival audiences. These events, away from the anxieties and pressures of the clinical environment, are key to discussing the personal effects of brain tumours on all aspects of the individual’s life. It is obvious from our discussions that patients embrace novel and bold research approaches to finding better treatments, and that they are very engaged in contributing to research. A patient suffering from treatment side effects and morbidity who is offered some hope and retains some positivity is better prepared for all forms of treatment. The prospect of telling patients that we are going to be able to better understand their brain tumour, and test drugs against it, may help to facilitate a more ‘positive mind set’, while delivering the herein described objectives. For example, many patients experience contributing tumour tissue (including our established theatre-to-lab research tissue

pathway) as a very empowering act in the face of a dismal prognosis.

**Species and numbers of animals expected to be used**

- Mice: 550

## Predicted harms

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

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

Our ultimate goal is to translate our most promising research findings into clinical applications that ultimately benefit brain cancer patients.

The mouse models described in this project are required for mimicking the human disease and brain tumour patient experience by integrating research before and after the tumour is resected by neurosurgery.

Considering animals at a more immature life stage: our project revolves around models of adult brain tumours. Approximately 93% of primary brain and central nervous system tumours are diagnosed in people over 20 years old with people over 85 having the highest incidence. The average age at diagnosis is 57. Therefore, this project and its protocols are designed to increase our understanding of the cells driving tumour growth in the fully developed (adult) brain, including tumour recurrence.

Considering species that are less sentient: our project fills a gap in modelling brain tumour biology in the central nervous system environment, which we currently cannot replicate with cell culture approaches as they do not sufficiently mimic the anatomy of the brain. The human brain is unique; however, there is a body of evidence showing that many important aspects including tumour initiation, development, and invasive growth of tumour cells into brain structures (for example, the corpus callosum, the cortex, the striatum, and the ventricles) can be soundly studied in the mouse brain.

Overall, in vivo brain tumour modelling is well-established in mice. As of November 10th, 2020 the search term 'brain tumours, mice' retrieved 25,205 entries in the pubmed database. In contrast, the search terms 'brain tumours, fish' and 'brain tumours, flies' retrieved 1065, and 456 pubmed database entries, respectively.
Considering animals that have been terminally anaesthetised: this can be considered whenever experimental endpoints are reached. It is however neither an option for the surgical procedure that is required to implant cancer cells into the mouse (with the purpose of initiating tumour development), nor for mimicking tumour relapse after surgical resection of the brain tumour. Moreover, non-invasive imaging procedures are required to monitor tumour growth, for example in presence/absence of a treatment strategy. All these procedures entail full recovery of the animals after they have been anaesthetised.

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

Typically, an experiment starts with the implantation of cancer (or control) cells (under anaesthesia) into the brain. Animals are anaesthetised by inhalation of anaesthetic agents and the head is fixed in a stereotactic apparatus. Analgesia +/- antibiotic are administered

prior to making a scalp incision. All surgical procedures are performed using standard sterile aseptic techniques. A small hole is drilled into the skull. Cancer cells are injected into the brain using equipment that allows us to deliver precise cell numbers and fluid volumes into the desired location. The needle is subsequently removed, the skull opening is sealed with bone wax and the skin incision is closed with tissue glue. Animals are observed in a warming chamber until full recovery from anaesthesia. After full recovery from the surgical procedure, the typical animal may experience several (non-invasive) imaging procedures that enable us to measure and predict the tumour growth in the animal's brain.

Once the tumour has grown, we may administer a substance (e.g., a chemical compound) to test whether this agent can reduce tumour size and spread and/or prevent further cancer growth in comparison to a control substance. This may require daily dosing of a substance (up to twice per day) depending on its half-life and other chemical properties.

Our project entails tumour excision surgery. Animals are anaesthetised and all surgical procedures will be performed using standard aseptic techniques. The previous incision from tumour implantation (see above) is reopened and a biopsy punch and fine suction tip used to take out a large part of the tumour, thereby mimicking the surgical technique utilised in human patients undergoing comparable brain cancer surgery. Animals are subsequently observed, and tumour relapse is measured by imaging (e.g., via small animal MRI) under general anaesthesia. At ~8 weeks post surgery, the animal is culled followed by whole brain retrieval for research analysis.

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

After tumour cell implantation, the animals are expected to recover quickly. Based on our experience, the animals show normal behaviour (no pain or distress) after full recovery from the procedure. The animals are monitored daily according to a scoring table that assesses the clinical signs that are associated with growth of the tumours as based on our pervious experience and observations. As a first sign of tumour burden, animals typically show slight under-grooming, which is often followed by an evolving hunched posture and slight reduction in activity. Whenever these moderate signs of animal distress appear, this will be reflected in the total 'score'. Reaching a pre-defined threshold means that the experiment will be ended as soon as possible. When reaching the first threshold, we will consider the the overall objective of obtaining sound results so that the animals are not wasted. In some cases, a prolonged period of tumour growth may be required, for example to determine how long a treatment can maximally keep the tumour under control. Under this scenario, the animals will be monitored up to three times per day to observe progression of under-grooming and hunched posture and appearance/accumulation of additional signs including reduced breathing activity, disorientation of movement, isolation, and increased periods of inactivity that all indicate an increase in animal distress. Any of observed signs will be noted, which counts for the overall score. We have pre-defined a threshold that, once it is reached, ensures immediate termination of the experiment regardless of any anticipated endpoint by human killing, before the animal experiences pain and suffering from the tumour burden.

Typically, animals show completely normal behaviour (score = 0) until the late stages of tumour growth. Once the critical tumour burden for causing symptoms is reached, the animal health score increases to greater 5 (e.g., reflecting slight under-grooming). A score greater 5 triggers twice per day monitoring. As soon as the animal reaches the next score

bandwidth (>15), the animal will be monitored 3 times per day. Once the score reaches the final pre-defined threshold (>25), it is removed from the study by humane killing, independent of any other factors.

Like in human brain cancer patients, tumour-removing surgery can have adverse effects. For a small number of animals this could mean short term experience of severe (neurological) clinical signs such as strong disorientation leading to immobility. Hence, animals will be closely monitored during and after the procedure (hourly) to ensure that the animal's recovery period (several hours) stays within the expected scoring threshold (≤ 25). Any animals that are immobile after the recovery period (score = 26) will be removed from the study by humane killing.

**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)?**

This project entails to protocols.

Protocol 1: moderate bandwidth (300 animals)

Protocol 2: severe bandwidth (200 animals). Based on advice from collaborators (with expertise in such protocol) and input from neurosurgeons carrying out this procedure in patients, we expect that a maximum of 5% of the animals (10 animals total) may transiently (for several hours) fall into the severe category (likely during the immediate procedure recovery period) before removal by humane killing. We would like to point out that, if the procedure is successful, the tumour removal is substantially reducing symptoms caused by the expanding tumour mimicking the situation in brain cancer patients after neurosurgery (the first line clinical treatment for brain tumours).
**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?**

Our ultimate goal is to translate our most promising research findings into clinical applications that ultimately benefit human patients. In this context we are pursuing three major (inter-related) research strands that aim to better understand as to why cancer cells

in the brain are adaptive and not all equal, why, and how brain cancer cells survive and grow despite therapy. We aim to find solutions as to how we can therapeutically target the tumour cells more efficiently so that their growth can be substantially halted. It is important to note that all the underlying biology posing these questions is very dynamic happening in space and time. This biology cannot be meaningfully separated from its location and anatomy of the brain. We do not have tools that allow us to study brain tumour development and recurrence exclusively in a human brain (non-invasively) or in human cell culture, and therefore, we need animals to study brain tumour biology at the molecular level, which can provide answers to our research questions. The mouse models described in this project are required for mimicking the human disease and there is a body of evidence indicating that these in vivo approaches yield meaningful results in terms of studying the complex adaptive growth and invasion of tumours into the relevant brain environment. Here, we mimic the brain tumour patient experience integrating models enabling research before and after the tumour mass is removed by neurosurgery. There is currently no replacement for the herein described pre-clinical orthotopic tumour models using mice.

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

We have considered cell cultures including three dimensional structures mimicking brain tissue - so called organoids. These 3D systems can enable the real-time investigation of brain tumour cell infiltration into the brain model. . This is a powerful tool, for example in the early stages of investigating a potential anti-brain cancer treatment, and for observing brain tumour cell behaviour and adaptive phenotypes during the highly dynamic brain infiltration proces  The organoid technology is scalable and can help to reduce the number of animal experiments, but it is not yet a replacement for the more complex procedures that mimic the brain cancer patient pathway required for this project.

**Why were they not suitable?**

Brain tumour organoid structures still lack the concerted organisation and anatomy of an animal brain. They could be an alternative for animal brain studies in the future; however, it has to be pointed out that the use of miniature organs (organoids) that would closely resemble an animal (or human) brain in organisation and function would also raise ethical concerns, especially if these structures would gain consciousness. Currently, this level of brain organoid development is not possible (far from reality).

A mouse brain is a suitable structure for studying brain tumour growth providing conclusions that can inform the human disease (for example, tumour migration from one brain hemisphere to the other). We are also able to mimic the first line treatment of the human patient pathway, which is surgical resection of the tumour. No current in vitro model can faithfully capture the complexity of the brain tumour microenvironment found in vivo providing the 3-dimensionality of the tumour and residual cancer- harbouring zones post-surgery.

**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?**

We have estimated the numbers based on our published previous work and our calculations need to consider that even control tumours derived from the same cell model source can show growth differences. Our project aims are to establish and demonstrate the utility of two complementary in vivo models and then investigate a significant difference in effect between these groups. We have considered that several substances (e.g., newly made analogues of the small molecule inhibitor KHS101) may be tested and/or validated (using different regimes) within the lifetime of this project. We estimated animal numbers needed to establish the tumour resection models (~50) and we need to allow for a general engraftment failure rate of ~20%, and failure of tumour recurrence of up to 50%.

The given total numbers are also based on currently funded work as well as an estimate of project requirements that may become relevant within the next 5 years due to future grant applications (some currently pending). For imminent work, we have calculated the average cell number expected for each tumour for establishing a recurrent tumour cell library, and the number of investigated tumour regions of interest and serial sections required to investigate biological replicates for each of the models used.

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

We used the Design Assistant from NC3Rs (and the EDA report) to calculate the numbers for the imminent project work (to be carried out within the first three years of the project).

We use up-to-date ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) to compare compatibility of our research design with the ARRIVE checklist of recommendations. Our study design also typically incorporates the use of both male and female animals in order to eliminate any potential sex bias in the research outcomes. Moreover, we base our statistical considerations and group size determination and randomization on the literature and previously-published work.

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

We have established (and are using) a comprehensive *in vitro* (cell culture-based) investigation system that enables the determination of a specific cellular behaviour in a controlled (simplified) environment before increasing the complexity by utilizing *in vivo* models. Whenever assumptions required for the calculations of animal numbers can not be faithfully made due to insufficient pervious experience (data) and/or published information, we carry out pilot experiments with the goal of filling the gaps leading to assumptions that can predict the statistical power/scientific soundness of the planned experiment.

Our project entails the generation of a tumour recurrence (cell and tissue) model resource that will be shared with the wider research community.

**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.**

Orthotopic tumour models are currently the most refined method to address tumour development in a location-specific manner as the tumours can grow in their typical environment. These cancer models are well established in mice and are required for biomedical research. To address tumour complexity, it is important that tumour migration and invasion patterns in the mouse model mimic the situation in patient tumours. Importantly, our patient-derived xenograft models reflect hallmark features of aggressive brain cancer including extensive migration of tumour cells along typical migration routes that are also found in the brains of human patients. Moreover, these models utilize cells derived from patient tumours and as such reflect well the heterogeneity of tumour profiles in different patients.

Discomfort and distress of animals are limited by providing a pain relief agent (analgesic; METACAM; effects typically lasting 24 hours) directly after the mice have been anesthetized so that they do not experience significant pain from the procedure once they awake from the narcosis. We will consider relevant refinement(s) of the surgical procedures and imaging procedures described in this protocol. Intracranial cell transplantations in mice will be performed under anaesthesia and pain relief medication will be given after surgery. Animal will be monitored daily and will be culled humanly when showing adverse effects or other signs indicative of toxicity.

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

Considering animals at a more immature life stage: our project revolves around models of adult brain tumours. Approximately 93% of primary brain and central nervous system tumours are diagnosed in people over 20 years old with people over 85 having the highest incidence. The average age at diagnosis is 57. Therefore, this project and its protocols are designed to increase our understanding of the cells driving tumour growth in the fully developed (adult) brain, including tumour recurrence. We aim to identify new treatment targets by ensuring that findings from the herein described animal models are relevant to patients.

Considering species that are less sentient: our project fills a gap in modelling brain tumour biology in the central nervous system environment, which we currently cannot replicate with cell culture approaches as they do not sufficiently mimic the anatomy of the brain. The human brain is unique; however, there is a body of evidence showing that many important aspects including tumour initiation, development, and invasive growth of tumour cells into brain structures (for example, the corpus callosum, the cortex, the striatum, and the ventricles) can be soundly studied in the mouse brain. Due to its anatomy, the mouse brain also serves as a suitable model organ for other brain diseases such as neurodegenerative disorders. It has the advantage that neurosurgical procedures that mimic the tumour resection therapy in human patients can be performed. As an in vivo model, mice also have the advantage of being bred in many strains, some of which maintain an immunodeficiency. This allows us to implant human cells -derived from patient tumours- into the mouse brain (forming the herein described xenograft tumours). In addition, there are mouse cell tumour models that can grow invasively in wild-type mice with an immune system, Hence, an investigation system can be established that complements the absence of immune cells in xenograft tumours and genetic and phenotypic differences in mouse compared to human tumours.

Overall, in vivo brain tumour modelling is well-established in mice. As of November 10th, 2020 the search term 'brain tumours, mice' retrieved 25,205 entries in the pubmed database. In contrast, the search terms 'brain tumours, fish' and 'brain tumours, flies' retrieved 1065, and 456 pubmed database entries, respectively.

Considering animals that have been terminally anaesthetised: this can be considered whenever experimental endpoints are reached. It is however neither an option for the surgical procedure that is required to implant cancer cells into the mouse (with the purpose of initiating tumour development), nor for mimicking tumour relapse after surgical resection of the brain tumour. Moreover, non-invasive imaging procedures are required to monitor tumour growth, for example in presence/absence of a treatment strategy. All these procedures entail full recovery of the animals after they have been anaesthetised.

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

Animals are monitored daily and their health status is recorded using an established animal welfare scoring system. During and after procedures, animals are closely observed to ensure full recovery as indicated by normal animal behaviour and posture. Pain caused by surgical procedures is managed by administration of analgesics. Non-invasive disease monitoring (via imaging) that requires fixation of the animals for a short period of time is carried out under transient anaesthesia.

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

We follow the latest version of ARRIVE (Animal Research: Reporting of In Vivo Experiments). Currently: The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, PLOS Biology; https://doi.org/10.1371/journal.pbio.3000410.

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

We stay informed though the NC3R website and are aware of the available 3R online resources. We also discuss advances relevant to our protocols with other groups locally and collaborate nationally.

We carefully assess the *in vivo* methodology in newly-published research papers to identify potential advances in our field. We are passionate about further developing animal-free investigation systems for brain tumour research and consider these approaches highly relevant for reduction of animal numbers. Our procedures are informed by clinical neurosurgery and we aim to mimic procedures as closely as possible in mice (including aseptic techniques, disease monitoring, and pain relief).

We attend and participate in local events organized under the umbrella of the Animal Welfare Ethical Review Body (AWERB).

**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?

# 21. Understanding and targeting the drivers of malignancy

**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**

Cancer, Tumour microenvironment, Imaging, Therapy

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

## Retrospective assessment

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

**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?**

This project aims to understand the molecular drivers of cancer development and how it spreads to distant sites within the body (metastasis). Using this information we will test potential new drugs and treatments for their ability to prevent cancer development and progression.

**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 it's aims and if not, why not?

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

**Why is it important to undertake this work?**

According to the latest statistics compiled by Cancer Research UK 367,167 people in the UK were diagnosed with cancer from 2015 to 2017 and unfortunately there were 166,533 deaths from cancer between 2016 and 2018 (http://www.cancerresearchuk.org). There is therefore a need to understand better the key molecular determinants of cancer progression and devise new more effective treatments to benefit patients with cancer.

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

The main outputs will be publication in peer-reviewed journals and dissemination to the scientific community at national and international conferences. In addition the group is actively involved in public engagement events and we will continue to work with our Public Engagement Manager and Patient Advocate groups to increase public awareness of our work and its implications for health. Certain elements of the research may also generate exploitable intellectual property that could lead to the development of new treatment strategies and we will work with relevant organisations to develop these.

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

The main impact of this work in the short term will be to the scientific community through an increased understanding of the basic biology governing tumour behaviour and identification of potential new treatments. In addition the project will provide added value to the translation of such findings into clinical benefit for patients with cancer. For example, there is much excitement around harnessing the immune system to treat cancer based around the considerable success seen with current immunotherapies. Immunotherapy uses our own immune system to fight cancer. It works by helping the immune system recognise and attack cancer cells. However, not all patients respond and our data suggest that when combined with an inhibitor of focal adhesion kinase (FAK), an important protein that controls the ability of cancer cells to grow, increases the effectiveness of these immunotherapies.
Based on work carried out by ourselves and others, the activity of FAK inhibitors when combined with immunotherapies are currently been assessed in patients with a number of cancer types. Current focus on FAK inhibition in glioblastoma, a very aggressive brain cancer, and its role in regulation of the immune system suggests a possible treatment opportunity in this difficult to treat tumour type that could be realised within the timeframe of this project.

In the longer term, we hope these studies will continue to inform on the biology of the cancers that we study and aid in the implementation of rationally designed forms of cancer therapies that more effectively treat the disease and provide benefit to patients with cancer. This is of particular importance for cancers where there are limited treatment options and prognosis is dismal such as pancreatic cancer, sarcoma and glioblastoma.

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

Findings from this project will primarily be communicated and disseminated through publication in widely-read peer-reviewed journals, but also presentation at local, national and international congresses and individual institute seminars. To ensure maximum dissemination, only journals with green or gold open access options will be considered. Furthermore, to expedite dissemination of knowledge, data will be published on an open access preprint repository such as bioRxiv.

I will continue to work closely with clinical collaborators to ensure the clinical relevance of our work and to rapidly translate our findings. Our previous track record in this area, where we have contributed directly to clinical trial design, demonstrates that this is an effective approach to maximise outputs from the project.

Any commercial outputs arising from the project will be managed by local commercialisation services. They provide support for industrial collaborations and commercialisation and will maximise commercial outputs of the project.

**Species and numbers of animals expected to be used**

- Mice: 37,500

## Predicted harms

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

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

The mouse as a model for human cancer research has proven to be a useful tool due to the relatively similar genomic and physiological characteristics of tumour biology between mice and humans. Mice have several similar anatomical, cellular, and molecular characteristics to humans that are known to have critical properties and functions in cancer and use of mouse models are key to understanding the molecular drivers of the disease. Use of adult mice is required to follow tumour progression.

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

Tumours will be initiated in mice either spontaneously, when using genetically engineered mouse models, or following injection or surgery, in transplantation models where tumour cells are directly injected into the mice. Effects on tumour initiation and progression following genetic or therapeutic intervention will be monitored. When testing therapies these will be administered via the most appropriate route and frequency for the individual therapy. Mice will often be imaged to monitor tumour development. This can occur up to twice weekly in the more rapidly growing transplantation models or monthly in the genetically engineered mouse models. Experiments using genetically engineered mouse models typically last from 6-12 months while those using transplantation models last around 4-8 weeks.

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

The development of tumours is associated with a number of adverse effects and is dependent on the tumour type:

Mammary Models

Mammary (breast) models may develop multiple tumours in the mammary fat pads. In the advanced stage, for a few mice, this may cause discomfort and hamper mobility.

Pancreatic Models

Animals developing pancreatic tumours may show signs of pain, such as hunching, piloerection (hair standing on end), lack of grooming and decreased activity as tumour development progresses.

Sarcoma Models

Sarcoma models may develop a variety of tumours including angiosarcomas (tumours that develop in the blood vessels), osteosarcomas (tumours that develop in the bones) and thymoma (tumours that develop in the thymus). In the majority of cases angiosarcomas will not cause any adverse effects; a very small number may ulcerate. Animals developing osteosarcomas may experience pain and decreased mobility, particularly if development is in a limb or joint. Thymomas may cause increased difficulty in breathing: labored breathing, shallow rapid breathing, gasping, or abdominal effort in breathing. This is generally only visually apparent in the advanced disease and is used as a study endpoint, therefore the duration of the effect on the animal is short. It may or may not be accompanied by a decrease in body score (Body condition scoring is a system approved by the AWERB for assessing the health of mice). Occasionally this development may not be outwardly apparent and can result in death. This is dependent on the model being used: thymoma development typically occurring in up to 10% of the total number of animals, with death due to thymoma occurring in up to 15% of thymoma-developing mice, 0-5% of the total number of mice, depending on the model.

Subcutaneous and Mammary Fat Pad Transplantation Models

Subcutaneous, and, rarely, mammary fat pad tumours may be subject to ulceration. If this occurs, the majority of mice will be terminated on appearance of ulceration, however some syngeneic tumour models (where tumours develop in mice following transplantation into mice with an intact immune system) may harbour ulcerated tumours for 2 – 3 weeks. Typically, mice do not display evidence of pain or discomfort due to the ulceration.

Metastatic Transplantation Models

Tumours which metastasise to the lungs may cause increased difficulty in breathing. This is generally only apparent in advanced metastasis development and is used as a study endpoint, therefore the duration of the effect on the animal is short. It may or may not be accompanied by weight loss. For a small number of animals this development may not be outwardly apparent and may result in death.

Carcinogenesis Models

Topical application of carcinogens may produce transient discomfort and pruritus (itchy skin), and development of papillomas (benign tumours that grow on the skin). A small number may have adverse effects, including persistent skin reddening or increased

irritation.

Glioblastoma Transplantation Models

Glioblastoma (brain tumours) models will exhibit neurological symptoms as the disease progresses. These can include ataxia (difficulties with balance), twitching, limb spasms, lack of a reach response, or general lack of grooming. These may be accompanied by a hunched posture and reduced movement, with or without weight loss.

Surgical Procedures

Mice undergoing surgical procedures may experience pain and exhibit short-term loss of appetite and weight loss post-surgery. Pain is managed by the use of analgesics and weight is generally regained within a few days.

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

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

Mammary Models

Mammary models are expected to be of mild to moderate severity. Approximately 30% of animals that develop tumours may experience mild severity with 70% moderate. >1% will experience noticeable discomfort and mobility issues.

Pancreatic Models

Pancreatic models are expected to be of moderate severity. >75% of animals developing pancreatic tumours will experience this level of severity.

Sarcoma Models

Sarcoma models are expected to be of moderate severity. >75% of animals developing sarcomas will experience this level of severity. A small number, cases in which thymoma development is not apparent, may be severe. This is strain-dependent, with death due to thymoma occurring in up to 15% of thymoma-developing mice, 0-5% of the total number of mice, depending on strain.

Subcutaneous and Mammary Fat Pad Transplantation Models

Subcutaneous tumour ulceration: ulceration may occur in approximately 50% of implanted tumours. Transient ulceration, which lasts for 1 - 2 days before mice are culled or the ulceration heals is expected to be of moderate severity. Prolonged ulceration, lasting up to 2 - 3 weeks, is expected to be of severe severity, due to the potential suffering caused by the ulceration, as yet unproven.

Metastatic Transplantation Models

Metastatic models are expected to be of moderate severity. Of animals implanted with tumour to model metastasis, e.g. intravenous, 95 - 100% will be of moderate severity, metastasis from implanted tumours up to 5% of the total number implanted, with <1%

severe.

Carcinogenesis Models

Carcinogenesis models are expected to be of mild to moderate severity with approximately 25% of animals experiencing mild severity and 75% moderate.

Glioblastoma Transplantation Models

Glioblastoma models are expected to be of moderate severity with >75% of animals experiencing this due to tumour development.

Surgical Procedures

Surgical procedures are expected to be of moderate severity for up to 100% of animals due to recommended severity levels for surgical procedures.

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

- Used in other projects
- Killed

**A Retrospective assessment of these Predicted harms will be due by 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?**
Tumours are comprised of multiple cell types and their behaviour is governed by the surrounding environment in which they develop. Currently there are no non-animal laboratory models that recapitulate this complexity and it is therefore necessary to use animal models.

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

Use of tumour cell lines and primary patient derived material.

**Why were they not suitable?**

Although important information can be obtained from the use of cancer cell lines using 2D and 3D models in the laboratory setting it is not possible to recapitulate the complex tumour environment.

The importance of the tumour microenvironment on tumour development and response to therapy highlights the need to use mouse models to progress these studies. In addition we

are interested in understanding the process of tumour initiation and progression through to metastatic disease and these temporal studies are only possible when using animal models.

The activity of drugs is governed by their distribution in the body and requires studies to be carried out in living organisms.

**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 estimated number of animals that we will use is based on previous experience of the different models that we use combined with statistical calculations and predicted usage based on current grant funding.

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

For experimental mice, experimental design will be kept as simple as practically possible, in order to maximise the information obtained from the minimum number of animals. This is based on statistical calculations to ensure experiments are sufficiently powered to generate significant results and use of the NC3Rs Experimental Design Assistant.

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

Based on our experience we have devised the most effective breeding strategies to generate mice that will develop tumours therefore reducing the unnecessary breeding of mice.

In some cases pilot experiments are undertaken to identify effect sizes and appropriate time points for analysis.

We contribute tissue to the Breast Cancer Now funded SearchBreast mouse tissue repository which provides tissue to other researchers.

**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 use a number of cancer models including genetically engineered mouse models and transplantation models.

- Careful monitoring of mice during tumour development and adherence to defined clinical endpoints by experienced researchers. Our experience of these cancer models and the clinical signs has allowed us to refine the endpoints used to ensure reproducibility between experiments, allowing us to reduce animal numbers, and ensure animal suffering is kept to a minimum.
- Careful monitoring of animals and use of analgesics following surgery.
- Use of environmental enrichment aids.

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

The mouse as a model for human cancer research has proven to be a useful tool due to the relatively similar genomic and physiological characteristics of tumour biology between mice and humans. Mice have several similar anatomical, cellular, and molecular characteristics to humans that are known to have critical properties and functions in cancer and are key to understanding the molecular drivers of the disease and it is necessary to use adult mice for these studies.

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

Examples of refinement procedures:

The syngeneic mouse cancer models (where tumours grow in mice with an intact immune system) that we have developed provide us with a unique opportunity to monitor the effect of the immune system on tumour growth and metastasis. As the immune system plays a critical role in tumour development and response to therapy these cell lines provide excellent tools that more faithfully recapitulate the tumour microenvironment. However, these models are highly aggressive rapidly growing tumours that are prone to ulceration and Protocol 3 is therefore rated severe as mice may harbour ulcerated tumours for 2 – 3 weeks. To minimise aggravation of the ulcerated tumours paper bedding is used and “dome homes” are used for environmental enrichment. We have implemented a scoring

system to better define robust end points for these models that are prone to ulceration that can be used by all staff and also provide images of ulcerated tumours as reference, which are displayed in the holding rooms. We continue to work with our colleagues to add to these as further animals develop ulcerated tumours. In our experience animals with ulcerated tumours continue to behave normally with little behavioural evidence of pain in the form of licking, reduced activity or food intake. This may be because the tumours are in non-weight bearing sites so there is no pressure on the tumour. However, mice are closely monitored. To investigate this observation further we will take steps (in conjunction with the named veterinary surgeons) to determine whether the animals are in pain by monitoring their behaviour/movement in the presence or absence of analgesics and also determine whether the use of analgesics is compatible with the scientific endpoints. It is worth noting that not all human ulcerated tumours are painful (http://www.cancerresearchuk.org/about-cancer/coping-with-cancer/coping-physically/ulcer/symptoms- of-ulcerating-tumors) and it is therefore important to try and establish whether or not this is the case in these models.

We have recently purchased an IVIS imaging system which allows us to monitor tumour development in our transplantation models using luciferase based (bioluminescence) imaging in live animals. This has helped in monitoring of brain tumours and can be used to cull animals in some experimental set-ups prior to development of clinical signs. In addition it has allowed us to follow metastatic spread to other organs in the body in our breast cancer models. To date the luciferase markers used have not been sufficiently sensitive to measure smaller metastatic tumours in live animals. However, more sensitive luciferase markers have recently been developed and the use of these will be explored.

We are continually working towards refining our monitoring of brain tumour development and are currently looking at the possibility of using microCT imaging to provide a more robust non-invasive read-out of tumour volume.

Recently intracaudal administration of tumour cells has been shown to be a reliable model of bone metastasis and provides a much more efficient and refined methodology for delivering tumour cells to the bone microenvironment. We will look at using this route of administration for our work on bone metastasis.

**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 (Br J Cancer 102, 1555-1577, 2010)

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

Through consultation of the NC3Rs website (https://www.nc3rs.org.uk), attendance at local annual 3Rs symposiums, attendance at the Federation of Laboratory Animal Science Associations Congress, reading the scientific literature, and interaction with colleagues and veterinary staff at the University and the wider scientific community. Any appropriate advances are implemented in consultation with the veterinary and technical staff at the University.

**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?

# 22. Ascertaining the links between the microbiome and innate immunity and how they may be used to combat the impacts of infectious diseases threatening amphibian biodiversity

**Project duration**

5 years 0 months

**Project purpose**

- Basic research
- Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work

**Key words**

amphibian decline, infectious disease, antimicrobial peptides, microbiome, conservation

| Animal types | Life stages |
|---|---|
| Alytes obstetricans | juvenile, adult |
| Xenopus laevis | 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.

- 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?**

In this project, we seek to develop an understanding of the interplay between amphibian immunity and the microbiome, and how these dynamics in turn affect, or are affected by, exposure to a potentially lethal pathogen or pathogens. This knowledge base will be developed with the intention of applying findings to both the management of captive amphibian populations and the mitigation of globally emerged infectious diseases that are drivers of the global amphibian decline.

**A Retrospective assessment of these aims will be due by 09 September 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Literally hundreds of amphibian species are at risk of extinction or have been driven to extinction by two classes of infectious agents, batrachochytrid fungi and ranaviruses. This in no small part is why amphibians are the most threatened vertebrate class: approximately 40% of extant amphibian species are considered to be at risk of catastrophic declines and possible extinction. Many of the threats behind this are amenable to interventions. For example, degraded habitat can be modified to benefit amphibians threatened by habitat loss. However, successful mitigation of the threat posed by infectious diseases has only been accomplished once, and then only for a single species on a small island. An international effort is underway to develop the strategies that support the effort to conserve amphibian biodiversity threatened by infectious diseases, either *ex situ* efforts in captive breeding facilities, or *in situ* efforts to mitigate the impacts in nature after disease emerges. We are part of that effort, and one our research efforts is focussed on manipulating the ability of amphibians to fend off infection through the production of antimicrobial peptides (AMPs, important components of the amphibian innate immune response) and the acquisition and maintenance of commensal bacteria, fungi and protozoa that persist on the skin and in the gut (the microbiome). To do this, we must undertake animal experimentation, and in this project we intend to use controlled experiments to ascertain how innate immunity and the microbiome interact and contribute to the probability of infection with and development of disease caused by batrachochytrids and ranaviruses.

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

We certainly expect this project to continue our record of publishing high impact, peer-reviewed literature that is used by many to develop their own research programmes and inform conservation efforts to mitigate disease. We expect more immediate and direct impacts on conservation programmes that are managing threatened species in captivity. Several of our study species are part of an international effort to assure the survival of species threatened by chytridiomycosis and ranavirosis through captive breeding. At least some of our experiments will involve dietary and environmental manipulations that we hope will inform how to manage captive settings for the benefits of immunity.
We also expect that part of the research outlined in this license will support research of post graduate students. We also have an ancillary program to constantly improve animal welfare in our research, which we do by embedding welfare research within our basic research experiments. For example, we are in the process of adopting video surveillance into at least some of the experiments we will undertake under this license.

Last, we are part of the IUCN's overall effort to aid conservation, and as such our work

provides crucial evidence that addresses several of the priority areas for research: i) Identify high-priority candidate microbial species that limit or prevent infections; ii) Support rescue pods for species with no other options; iii) Comparative studies of species/population susceptibility (tolerance/resistance) in key species in particular regions using common experimental design, iv) Conduct surveys of immune defenses in areas not surveyed and assess susceptibility of priority species

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

The first beneficiary will be the academic community. Research on microbiomes and immunity is extremely topical and appeals to an enormous audience. More specifically, research on amphibian infectious diseases for the purposes of conservation has grown exponentially since the early 2000s. The relationship between amphibian immunity and microbiome is largely unexplored, yet both of these have been identified as key players to be investigated as targets for disease mitigation. We expect to publish our research in a prompt fashion, for which we have an extensive, proven track record. We also have a track record of embedding post-graduate research students and other early career researchers in our projects, some of which are already involved in the development of this plan of work. The broader amphibian conservation community uses the Amphibian Conservation Action Plan (ACAP) as a guideline for targeting research and informing policy, and new findings will be used to update this document.

We expect to directly inform conservationists who manage captive populations of disease-threatened amphibians, a global network that coordinates the captive management of hundreds of amphibian species, some of which are target species for this project. Through our ongoing partnerships with zoo staff and curators responsible for amphibian management, we can ensure that relevant outputs can become part of policy documents, and where appropriate, the husbandry guidelines for specific species.

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

We have a track record in communicating findings through publications, conservation management and policy documents and webpages. We will also seek to hold a 2 day symposium with associated workshops. Our last such event brought nearly one hundred specialists from around the world and convened two ancillary workshops.

Our welfare work directly informs best practices for our own experiments. In addition, we will be discussing convening a follow-up meeting for our initial workshop on welfare in amphibian research, outlining new developments and sharing outputs that have addressed some of the concerns we outlined in the companion publication for the workshop.

Our team is networked globally and works with comparable teams across Europe, the Americas, Africa, Asia and Oceania. We communicate regularly with other teams, sharing prepublication findings and aiding in the global effort to combat threatening infectious diseases of amphibians. These informal lines of communication are a key route of communication, and we have already informed colleagues we are investigating these topics; in no small part the reason we are submitting a joint NSF/BBSRC application with US American colleagues, who, in fact, attended the symposium.

**Species and numbers of animals expected to be used**

- Other amphibians: No answer provided

- Xenopus laevis: 70

## 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 European amphibian species that are threatened by batrachochytrid fungi and ranaviruses. Several are part of *ex situ* captive breeding efforts to assure survival in the face of extinction risk due to infectious diseases. This work involves both larval and post-metamorphic stages (juveniles and adults) as mortality due to ranaviruses is occurring in nature that affects both of these stages. As well, larvae act as reservoir and amplification hosts for batrachochytrid infections, which can then cause mass mortality near the end of or after metamorphosis is complete.

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

We will typically house animals individually, but sometimes in groups, and allow microbiomes to form from what is available in the environment. In some circumstances we may intentionally alter environments to provide different microbial communities from which microbiomes are recruited, or apply potentially probiotic and unharmful microbials to animals. During this period, we may alter diets to determine if this has any effect on the production of AMPs or microbiome composition. Animals may then be exposed to batrachochytrid fungi, ranaviruses or in some circumstances both, or combinations of pathogen genotypes. We may sample microbiomes and AMPs preceding pathogen exposure, or after, depending on the needs of the individual experiment. We may also alter housing arrangements, for example switch individually exposed animals into group settings to track transmission dynamics, alter diets to see if this has any effect on AMPs, microbiomes or infection dynamics, or environmental conditions to see how this affects any of these factors.

The majority of these experiments will last a matter of weeks, possible two or 3 months. Welfare is tracked through daily checks, and we may trial the utility of video surveillance to monitor welfare.

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

Chytridiomycosis caused by *Batrachochytrium dendrobatidis* typically affects only post-metamorphic animals, but we will monitor exposed larvae for any indirect effects of infection, such as slow growth, inappetance or abnormal behaviour. Post-metamorphic amphibians may experience inappetance, apathy, immobility and poor limb movement. Death arises from electrolyte imbalance that causes heart arrhythmia and eventual failure. We have humane endpoints in place to ensure we can intercede before disease has progressed to the point of arrhythmia.

Ranavirosis caused by ranaviruses also affects larval forms as well as post-metamorphic animals. Viruses cause cells to rupture (apoptosis) leading to tissue damage that can affect most organs.

Externally, disease manifests as ventral reddening of limbs, petechial haemorrhages under the skin surface and superficial skin blisters and sores on various locations, often the tips of digits, the underside and insertions of limbs and around the eyes. Internal lesions and haemorrhages can occur in many internal organs, and commonly affecting the liver, muscle, kidneys, digestive tract and reproductive organs. Here we also have endpoints in place, and have a researcher currently working towards improving these, as despite what we already know about ranavirosis, disease progression can be extremely rapid and progress from no visible signs of disease to death in less than 24 hours.

None of the protocols we have in place for modifying diet, housing, swab sampling for AMPs, microbiomes and pathogens or any other manipulations cause any prolonged stress or cause pain, suffering or distress.

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

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

All experiments restricted to batrachochytrids should be considered moderate, as none of the procedures and protocols associated with this one pathogen cause pain, suffering or harm, and we intervene long before disease progresses to the point of serious ill health and all mortality will arise from humane killing. Experiments involving ranaviruses do generate some mortalities that can be directly attributable to disease, and humane endpoints we rely on include external manifestations of ranavirosis that likely cause pain and discomfort (e.g., superficial skin blisters and sores). In our experience fewer than 5% of animals we expose to ranaviruses fall into these categories, as we enact interventions (euthanasia), whenever possible, days before when we predict these external signs manifest.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 09 September 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?**

As of now, we have no replacement models that can mimic the recruitment of microbiomes, the expression of antimicrobial peptides and how these interact with either a

dermal (batrachochytrid) or systemic (ranavirus) infection

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

Tissue/organ culture and organ-on-a-chip. We have completed a first assessment of organ culture as a means of looking at infection alone.

**Why were they not suitable?**

We are years away from developing tissue/organ culture and organ-on-a-chip models that can replicate the core components of the antimicrobial peptide/microbiome/pathogen interface. We will continue to explore these options, but the urgency of the conservation issue requires immediate investigation.

**A Retrospective assessment of Replacement will be due by 09 September 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

**How have you estimated the numbers of animals you will use?**
We have selected sample sizes based on our previous work on survival (see below and power analyses) and field studies of peptide production in one of our focal species. Total numbers vary across species based on specific questions. Smaller sample sizes are for species that we will only sample for peptide work. Larger numbers reflect use in experimental protocols where pathogen exposures may be undertaken.

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

Preliminary field data investigating antimicrobial peptide production and microbiome structure across populations of Alytes obstetricans has shown that 30 reference animals are sufficient to provide a baseline to which experimental animals can be compared to. For phylogenetic comparisons across species, we have set sample sizes to slightly more than double this in case work requires repeating. For experiments involving pathogen exposure, we do not have an understanding of what sample sizes are required to detect significant differences in peptide production or other measures of host and microbiome responses. Instead, we have relied on power analyses using data from previous pathogen challenge experiments using Alytes muletensis. From these we have concluded that 20 animals per treatment will generate significant differences (p=0.05) between treatment groups for survival when power is set at 0.8. Thus, for species where we have requested to use 120 animals, these represent sampling for both the phylogenetic component and basic

challenge work with one pathogen. Species where we have requested up to 400 individuals are ones where we expect to investigate host response interactions under different environmental conditions.

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

Where possible, we are sourcing animals from captive breeding projects that generate excesses that are typically culled. See above for examples of how we have estimated power and sample sizes based on previous field and lab-based studies.

**A Retrospective assessment of Reduction will be due by 09 September 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

The PI has greater than 20 years of experience undertaking disease experiments and within the facility we have 15 years+ experience working with 3 of the focal species. Together, we have established humane endpoints for Rana and Alytes that are directly transferrable to the other species, or have sourced data on captive management, welfare and when to intervene using the management plans for several of our study species. In studies where the objective is to collect skin peptides only, we have ethically reviewed the use of norepinephrine baths and already trialled them in the field with one of our study species and in several neotropical amphibians. The student who will be responsible for collecting peptides has been trained in the method and his training approved by our NTCO. We expect no welfare issues with norepinephrine treatments.

In procedures which involve pathogen exposures, we have extensively investigated the progress of disease associated with both chytrid fungi and ranaviruses. As a result, we have clearly identifiable humane endpoints and strategies in place for enacting humane killing for anuran amphibians at early stages of the infection and disease process to avoid animals experiencing any unnecessary suffering associated with infectious disease. These have already been applied successfully to 3 of the study species we propose to use in experimental settings under other PPLs and are used as standard welfare indicators on an even wider range of amphibians by the research community.

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

We cannot do this work on larval forms, as the full complement of skin secretions are not produced during the larval period. Glands responsible for secreting peptides are fully formed only after metamorphosis is completed. What little information that is available on anaesthetics on amphibian skin has revealed that anaesthesia can cause skin damage. Glands for peptide production are embedded in the skin, so we are concerned that anaesthetics would reduce the likelihood the protocol would yield the desired results.

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

We are already trialling the use of video surveillance for detecting humane endpoints. In the event the results show that this improves welfare under the experimental settings we propose to use here, we will implement video surveillance.

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

We use ARRIVE guidelines for reporting experimental results. Unfortunately there is a distinct lack of published guidance specifically for refinement in experiments involving amphibians, but we do rely on the governmental guidelines available to us at https://www.gov.uk/guidance/research-and-testing-using- animals.

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

We have one PhD student actively working on welfare indicators for amphibians in the context of disease research and experiments. She works closely with our NACWO and our group is extremely active in communicating findings. her work already directly informs our programme of work and any new applicable findings will be embedded in our work and this project license will be amended to reflect this. We also work closely with the European Xenopus Resource Centre (EXRC) and are applying their up-to-date standards to our Xenopus work.

**A Retrospective assessment of Refinement will be due by 09 September 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?

# 23. Paediatric airway surgery training model

**Project duration**

5 years 0 months

**Project purpose**

- Higher education and training

**Key words**

Surgical training, Otolaryngology, Paediatric Airway Surgery

| Animal types | Life stages |
|---|---|
| Rabbits | 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.

- Education and training licence

## Objectives and benefits

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

**What's the aim of this project?**

This project aims to deliver world-leading training in paediatric (children's) airway surgery (PAS) for the first time in the UK. This will provide senior Otolaryngology (ENT) trainees aiming to become Paediatric Airway Consultants with the skillset they need, without having to travel abroad to obtain such expertise, as well as enable General ENT consultants to keep their out-of-hours emergency skills up to date.

**A Retrospective assessment of these aims will be due by 27 July 2026**

The PPL holder will be required to disclose:

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

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these**

**could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

Live surgical training in how to look after and manage the paediatric airway is considered vital to providing a safe emergency service to the UK population, as no other simulation model currently available provides the same level of technical fidelity and feedback. In addition, those surgeons specialising in Paediatric Airway Surgery require experience in additional advanced procedures prior to commencing surgery in humans. Clinical experience in these procedures is scarce by the nature of the set-up of UK regional surgical training, and it is therefore vital for safe provision of specialist services to incorporate other modes of surgical learning, including simulation.

**How will course attendees use their knowledge or skills in their future careers?**

There are two distinct cohorts of delegates who must demonstrate competency with PAS:

- ALL ENT trainees and consultants who undertake out-of-hours work who must maintain ‘emergency-safe’ endoscopic and paediatric tracheostomy skillsets. These surgeons will use their skills learnt and practiced on our course to crucially supplement their experience in dealing with the (thankfully infrequent) stressful paediatric airway emergency.
- ENT trainees and consultants aiming to subspecialise in PAS who require additional advanced practical knowledge of the open airway procedures to be performed. These surgeons require the knowledge and skills gained here as 'entry-level competence' to build their foundation for further clinical exposure in advanced PAS.

**What are the principal learning outcomes from the course?**

- Improved endoscopic competency of individuals to perform PAS procedures, as demonstrated by observed improvements in competence in paediatric bronchoscopic airway techniques.
- Improved surgical competency of individuals to perform PAS procedures, as demonstrated by observed improvements on cadaveric studies following completion of endoscopic tasks.
- The primary learning outcomes for this licence pertain to the acquisition and development of practical (PAS) skills by ENT trainees and consultants. The development of delegates’ skills over the duration of the course will be continually assessed by Faculty members to ensure that this output is met; this method of technical skill assessment closely mirrors current UK best practice training of surgical trainees in clinical practice by Consultant Trainers. Assessment will be a combination of self- and observer-based feedback.
- Improved confidence to manage basic paediatric airway emergencies in terms of both technical and non-technical skills

Through the other parts of the course that do not involve live animals, attendees will observe and then practice leadership in airway surgery scenarios. As role play of stressful scenarios is an increasingly recognised component of surgical training, this will be a third pivotal learning outcome in successfully getting to the stage of deploying their improved surgical technical skills.

**How are these learning outcomes important to the people on the course?**

Clinical Need for Paediatric Airway Surgical Expertise

In the paediatric population, improved prenatal and neonatal care has led to the survival of an increasing cohort of children with severe airway birth defects or extreme prematurity requiring prolonged intubation (Zhang et al, Sem Fetal & Neonatal Med 2016). There has also been an increase in survival of children with congenital syndromes, many of which have laryngotracheal manifestations or require tracheostomy for respiratory disease management. The coexistence of airway disease in paediatrics can greatly impact their quality of life by compromising breathing, swallowing and speech, and can complicate their care of other conditions. Each affected child can require multiple surgical procedures to correct or dilate their scarred or narrowed airway as they grow (sometimes every few weeks or months at the beginning of life), and some eventually require extensive laryngotracheal reconstruction or resection of diseased segments of trachea (Bailey et al, Eur Arch ORL 2003).

As a result of this explosion in the workload and complexity of Paediatric Airway Surgery (PAS), there is a rapidly-growing demand for all Ear, Nose & Throat (ENT) surgeons to be emergency safe in the management of the paediatric airway, and for a subspecialist group of surgeons able to perform PAS to correct such defects.

Despite the pressing need for the development and maintenance of PAS competencies amongst ENT surgeons, and the widely-acknowledged benefits of simulation in surgical training, and the fact that attendance on a high-fidelity PAS course is mandatory for subspecialisation credentialing in PAS, no course currently exists in the UK. Having a domestic-run course will greatly benefit Trainee course attendees who intend to specialise in PAS as they will be able to more easily afford to complete their training, and will benefit Consultant attendees who might otherwise opt for more convenient and costeffective methods of keeping their on-call PAS skills nominally in-date with simulation of lower fidelity and efficacy.

**Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?**

The primary benefit is hoped to be to patients, who will be treated by more confident and competent surgeons as a direct result of course attendance. However, the degree and timescale for this is difficult to quantitatively define, and therefore the most direct beneficial output will be in the acquisition and improvement of delegates' surgical and nontechnical skills in managing PAS.

The technical expertise and techniques of the surgeon are vital factors in the overall success of the treatment strategy, as are team communication and decision-making skills. It is therefore vital adequate training and rehearsal are undertaken. The incorporation of simulation training into Continuing Professional Development (CPD) in surgery has several purposes which all directly improve patient care:

- supporting learning and promoting surgical excellence by keeping up to date with technical advances;
- addressing specific learning needs (e.g. acquisition of new skills);
- situational awareness of human factors which can influence people and their behaviour, both as individuals and within teams;

- enabling practice and refinement of clinical skills without exposing patients to undue risk whilst the surgeon is learning.

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

After the inaugural pilot course has taken place, we will look for formal accreditation with the Royal College of Surgeons of England, which will greatly help our dissemination of the course's presence both domestically and internationally.

From the first course, we will also be recruiting Visiting Faculty members from other international PAS centres across North America and Australia. This will further increase local expertise, enable dissemination, collaborative improvements in how simulation training is best performed and kept as high-fidelity as possible.

**Species and numbers of animals expected to be used**

- Rabbits: 85

## 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.**

New Zealand White rabbits of around 3kg in weight have airways of comparable dimensions and shape to human babies and have little individual variation in tracheal diameter, making it straightforward and reliable to plan which age and weight animals are required for optimal surgical training. We have extensive experience in using the New Zealand White rabbit as an in vivo model and have found it to be an excellent simulation in terms of face and content validity to the clinical scenarios encountered in caring for babies and young children.

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

Animal suffering will be minimised by having all procedures performed under a single period of terminal anaesthesia without recovery. The depth of general anaesthesia will be continuously monitored throughout to ensure that there is no recovery of awareness. Animals will not be recovered for reuse under any other Protocol.

Once under general anaesthesia, course delegates will work through a pre-defined and supervised series of nontraumatic anaesthetic, endoscopic and minimally-invasive techniques that form the paediatric airway surgery repertoire, under the guidance of 1:1 instruction of trainers extensively skilled in human/rabbit paediatric airway surgery. This will include a combination of the following procedures, depending on the delegate's learning needs and speed at which they reach competence:

- Endotracheal intubation and/or veterinary laryngeal mask airway (VMA) placement.);
    1. Endoscopic (camera) diagnostic airway procedures
    2. Examination of the larynx, trachea and bronchi Secretion management and suction
    3. Airway foreign body retrieval (following placement of the foreign body by a

member of Faculty).

- Endoscopic balloon dilation of the airway;
- Airway stent placement and/or removal;
- Superficial tissue biopsies using endoscopic biopsy forceps;
- Endoscopic suture repair of small (less than 2cm length) tracheal defects (created immediately prior to the repair by a member of Faculty).

1. Management of airway bleeding using cautery, suction and/or topical adrenaline.

Animals will then be euthanised by deepening of the general anaesthesia to become terminal anaesthesia, and confirmation of death will be performed by a competent person as per Schedule 1 methods. The animals' cadavers will then be used to teach delegates open, more invasive, surgical airway procedures.

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

All procedures will occur under a single episode of non-recovery general anaesthesia, therefore adverse effects that the animal will be aware of solely relate to any transient mild pain or discomfort from the induction of the general anaesthesia via intramuscular injection. Animals are not expected to show any signs of suffering after this.

We stress that this live animal training sits as part of a longer 3-day course involving lectures, video streaming of live clinical operating, human factors (non-technical) simulation and plastic mannequin simulation. Training in live animals will therefore be kept to the absolute minimum that still results in adequate training of ENT surgeons to manage airway emergencies in children.

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

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

All animals will be non-recovery.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 27 July 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

The small dimensions of child-sized animal models render it difficult to get the same tissue feel in a cadaveric specimen, as management of secretions and bleeding are more relevant to a successful surgical outcome than in larger animal species and adult humans. We consider live animal use to be appropriate and unavoidable for optimal training in this particular circumstance.

**Why can't your aim be met by observing or by participating in ongoing research or clinical procedures?**

There is a rapidly-growing need for **all** Ear, Nose & Throat (ENT) surgeons to be emergency safe in the management of the paediatric airway, and for a subspecialist group of surgeons able to perform PAS to correct such defects.

On completion of training, almost all ENT emergency cover outside of specialist paediatric hospitals is shared amongst a hospital's ENT Departmental consultant body, many of whom do not perform paediatric ENT surgery as part of their routine elective work. As the above skills are not often called upon out-of-hours, consultants on general ENT on-call rotas run the risk of deskilling or becoming out- of-date in the management of basic PAS emergencies (e.g. inhaled foreign bodies, neonatal and infant stridor and paediatric airway obstruction necessitating tracheostomy) when they do arise.

Advanced paediatric airway surgical units are limited in number and localised to tertiary children's hospitals in most parts of the UK (with 1-3 units associated with each ENT geographical training area). In order to ensure that all trainees pass through a PAS unit, and because this represents just one ENT subspecialty of many in which trainees must demonstrate competence as a 'Day One' consultant, almost all paediatric ENT clinical placements are necessarily curtailed to 6 months in duration. As a result, despite being encouraged to develop this area of special interest, trainees intending to pursue subspecialisation in PAS are unlikely to be competent to take on PAS work at CCT without supplementing their training with considerable further experience in the form of domestic and overseas Fellowships; trainees must usually attend a live airway course such as this one in order to be allowed to operate by consultant trainers.

It is not practical, given that there are very few animal licences granted for airway surgical research in the UK, to suggest that all ENT surgeons and trainees will have the opportunity to get sufficient hands- on experience to be clinically competent by participating in live animal research.

**A Retrospective assessment of Replacement will be due by 27 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design**

**studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

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

We anticipate this course to run on an annual basis. Course delegate numbers (maximum 16 per course) and starting level of surgical competence will be tightly controlled to ensure a minimum number of animals are used. A single rabbit will be used per delegate, with a spare rabbit per course available in case of sudden unexpected death of an animal due to anaesthetic complications prior to any meaningful training objectives being met.

**What in silico or ex vivo techniques will you use during training?**

Over the first 2 days, delegates will first receive teaching and training using a variety of expert lectures, live-streamed demonstrations of procedures being performed in human patients, low-fidelity (plastic mannequin) models and non-technical skill (human factors) training. This not only reduces the need for live animal use, but is also more effective in developing a 'multi-layered' understanding of the intricacies, planning and management of bronchoscopic follow-up and/or treatment. This will also ensure individuals aiming to perform human surgery have practiced for likely pitfalls and complications ina simulated multidisciplinary setting (including anaesthetic and scrub nursing roleplayers).

**Will these techniques reduce animal numbers? If so, how?**

The animal work covered by this licence sits as half of the final day of a three-day multi-modal advanced paediatric airway surgery (PAS) course.

There are therefore multiple interlinked learning points that we plan to address over the course the three days, only the **penultimate** of which requires live animal use for maximum efficacy:

- Knowledge of the indications and steps of each procedure (lectures)
- Visual demonstration of the desired procedure in the clinical setting (live-streamed clinical operations)
- Acquisition of manual/technical skills and the deployment of endoscopic equipment and camera stack systems (plastic mannequins)
- Managing and anticipating complications within a multi-disciplinary team setting (non-technical group simulation)
- Application of endoscopic technical skills within a high fidelity simulated surgical environment (live animal procedures covered under this license)
- Application of open surgical technical skills within a high fidelity simulated surgical environment (cadaveric studies on the terminated animals)

**What other measures will you use to minimise the number of animals you plan to use in your project?**

The riskiest part of animals studies on other internationally-run airway courses is the open surgical work, generally involving the harvesting of rib grafts. This work would likely require more animals given the high likelihood of complications and need for early deepening of terminal anaesthesia. We will therefore perform this part of the course on the animals post-mortem, which significantly de-risks the procedures undertaken by the animals and gives

us confidence that each course delegate will be able to perform all required procedures on a single animal.

Course learning objectives will be tightly controlled to allow nontraumatic skill acquisition, followed by complementary surgical procedures, to be practiced sequentially on the same animal under the same period of non-recovery anaesthesia. To aid in efficient delegate learning consolidation, delegates will operate in pairs acting as both principal and assisting surgeons, under close on-to-one supervision from personal licencees and human paediatric airway surgical consultants. Delegate numbers and starting level of surgical competence will be tightly controlled to ensure a minimum number of animals are used.

**A Retrospective assessment of Reduction will be due by 27 July 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

The New Zealand White rabbit model is the best choice for modelling airway surgery in children, because they have large airways (voicebox and windpipe) of very similar dimensions and shape to human babies. There is also very little variation in airway diameter between individual animals of a given weight, making it straightforward and reliable to plan which age and weight animals are required for optimal surgical training, and minimising the chance of an animal being used which subsequently proves not to be suitable once it is examined under anaesthetic.

The Toronto airway surgery course employs live cats and pigs, without a cadaveric component. We feel that all procedures performed there on cats can be optimally performed here on NZW rabbits. Whilst piglets may be easier, and lower risk, for the performance of open airway surgical procedures than the smaller rabbit, we feel that there is little additional training benefit to performing these on a live animal. In cadaveric studies, the advantage of using a piglet over a rabbit extends only to the harvesting of adequately thick cartilage for shaping laryngeal grafts – this part of the procedure can be adequately modelled using cadaveric human cartilage, which we will provide. Thus, all training objectives can be satisfactorily met within the rabbit.

We are using procedure-naïve animals under a single episode of nonrecovery general anaesthesia. This therefore represents a model with the lowest possible pain, suffering and distress to the animals.

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

We are using animals that are terminally anaesthetised.

The rabbit is the minimum size species that can be used for simulating airway surgery in children – rats and mice have extremely small airways and would require microsurgical skills not relevant in the clinical setting, in addition to the fact that any bleeding or secretions in the airway would lead to the possibility of the animal dying prematurely during the simulation from airway obstruction. Other centres around the world offering comparable courses use pig or cat, both of which we feel to be unnecessary to meet the same learning objectives.

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

Animals will receive a seven-day acclimatisation period in the animal unit, where they will be group housed with appropriate food and environmental stimulation where possible.

Animals will be monitored continuously throughout the anaesthetic to ensure that there is no chance for recovery of awareness. Despite the fact that all procedures will be carried out under a single episode of non-recovery general anaesthesia, painkillers will still be administered to all animals at anaesthetic induction.

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

We will follow the advice and guidance of the LASA anaesthetic guidelines for rabbit anaesthesia. We will also follow the Association of Surgeons in Training (ASiT) guidelines for best practice in surgical simulation.

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

Our work will be continuously monitored by our unit's Named Veterinary Surgeon and we will be in conversation with the UCL AWERB throughout. We will also subscribe to the 3Rs newsletter so that any advances in rabbit anaesthesia or other relevant 3Rs topics that can improve the welfare of these animals will be acted on immediately.

**A Retrospective assessment of Refinement will be due by 27 July 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?

# 24. Assessing freshwater fisheries

**Project duration**

5 years 0 months

**Project purpose**

- Basic research
- Protection of the natural environment in the interests of the health or welfare of man or animals
- Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work

**Key words**

Fisheries management, Salmon conservation, Trout conservation

| Animal types | Life stages |
|---|---|
| Atlantic salmon | 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.

- Uses endangered animals

## Objectives and benefits

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

**What's the aim of this project?**

The aims of the project are to provide information on numbers of salmon, trout and eels in selected UK waters and the responses of these fish populations to changes in their environment. This is to inform fisheries management and conservation.

**A Retrospective assessment of these aims will be due by 19 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 it's aims and if not, why not?

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

**Why is it important to undertake this work?**

The aquatic environment is undergoing rapid change from a wide range of factors, such as climate change, and more localised variations associated with industry and farming. Salmon and trout are emblematic of good habitat quality, support valuable fisheries, and are of noted conservation importance. The European eel is now endangered and requires particular close study to develop suitable conservation measures. Knowledge of the ways that individual fish and their populations respond to environmental changes is required to develop appropriate mitigation and conservation strategies for these species. The information is needed to conserve fish populations and allow for effective management of the fisheries that exploit them, and mitigate against other impacts resulting from human activities such as aquaculture and climate change.

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

The work will maintain long term datasets examining specific fish populations (e.g. salmon, sea trout and eels). In addition, novel studies will be conducted to examine specific environmental and management challenges to fish populations. These include: assessments of population size to determine their sustainability, tracking studies to understand migratory behaviours in relation to potential hazards, studies examining the influence of environmental factors such as temperature on fish numbers, and genetic studies to aid conservation measures. Together this work will generate various outputs including scientific publications, briefings, policy documents and contributions to national and international bodies regarding fisheries management and conservation.

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

The information obtained from the work is needed to conserve fish populations and allow for effective management of the fisheries that exploit them, and mitigate against other potential impacts resulting from human activities such as aquaculture and climate change.

While some of the outputs will have impact in the short term as they are produced, informing management and contributing to the scientific knowledge base, other data collected will contribute to building long term datasets allowing analysis of fish population trends over decades. This type of data collection is required for understanding fish population responses to environmental changes.

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

Outputs will be maximised by publication through scientific journals, presentation at conferences and in discussion with relevant policy and stakeholder groups. These groups include fisheries and rivers trusts that are directly involved in fisheries management, government and national and international organisations concerned with salmonid conservation.

**Species and numbers of animals expected to be used**

- Other fish: No answer provided

## Predicted harms

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

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

The fish species used are those that are of management and conservation importance.

The work will focus on juveniles and adults as these are the life stages that can most readily be used to determine the status of the fish stocks.

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

Typically studies will involve counting and measuring fish collected from the wild to assess population size and structure.

Some fish may have a small quantity of scales removed to assess age and growth responses to changes in temperature or environment. In addition some fish may have small piece of fin removed to examine their genetics.

Studies examining the behaviour, survival, or growth of fish may require the fish to be tagged. Tags may be attached externally or internally (eg a PIT tag).

All fish will be handled under mild anaesthesia before being released back into the wild. While dependant on population sizes, it is estimated that up to 123,000 procedures will be conducted over the 5 years.

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

It is expected that the impacts on the fish will be minimal. Occasionally individual fish may be damaged, or killed during capture. Fish identified with severe skin or fin damage, or displaying impaired swimming behaviours will be killed by a schedule 1 method as a humane endpoint. Since the work is to monitor populations and biology of fish in good condition, every effort will be made to use the least adverse methods available to obtaining data.

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

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

The majority of procedures performed, such as measuring length, will be mild (>95% fish).

Where internal tags are used the expected severity is moderate as a level of discomfort may be expected to occur on recovery from anaesthetic (5<% fish).

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

- Set free

**A Retrospective assessment of these Predicted harms will be due by 19 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?**

The work involves counting wild fish in natural systems to assess population sizes, collecting genetic and scale data, or observing their behaviour. In performing this work there are no alternatives other than to use fish.

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

Mathematical modelling approaches and automatic fish counters can be used to estimate fish populations.

**Why were they not suitable?**

These approaches are already used, as appropriate, in the programme of work described. Data obtained from fish collected in the wild is required to input into models to make predictions and aid in model development. Automatic fish counters are present in a number of rivers but they are large fixed structures that provide data in a limited area. They do not provide key biological data such as growth rates that can only be obtained through handling a fish.

**A Retrospective assessment of Replacement will be due by 19 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 yourexperience?

## Reduction

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

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

The workplan represents a continuation of fish population assessments, and therefore the estimates regarding numbers of fish are based on the experiences of previous years.

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

The numbers of animals studied are minimised by targeting specific study sites to provide suitable indexes of population health. Similarly, behavioural studies will use only the number of fish required to identify the scale of any effect of habitat change, however, certain monitoring work requires an entire population to be sampled.

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

In the case of experimental work we adhere to the principal where existing pilot work will be used to estimate an appropriate sample size to identify effects, typically at the 95% level of confidence. Advice will be sought, as required, from professional statisticians with regard to numbers of animals to be used.

**A Retrospective assessment of Reduction will be due by 19 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.**

The species used are those whose populations need to be assessed. The proposed work is primarily focused on Atlantic salmon and brown (sea) trout, which are the species of most importance in supporting fisheries.

After capture, fish will be anaesthetised to minimise distress during their time out of the water. They will be allowed to recover from the anaesthesia before being assessed as behaving normally and being returned to the water they were taken from.

Since the aim of the work is to monitor populations and biology of fish in good condition, every effort will be made to use the least adverse method available commensurate with obtaining required data.

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

Less sentient, immature alternatives would be at the alevin stage of fish development. These stages would be very susceptible to damage if handled and would not provide the data required. The work is focused on assessing fish populations and this requires study of juvenile and adult fish.

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

Fish will be handled under mild anaesthesia to minimise stress. Gentle handling and aseptic technique will be used to minimise the possibility of secondary infection. All efforts will be made to use the smallest tags possible while allowing the required information to be obtained.

Fish will be monitored to determine their recovery and assess their well-being post capture, and prior to release.

All staff involved in the procedures are skilled in fish capture and handling techniques using defined protocols. Accurate records, including comments/ observations are made allowing for review of procedures. Procedures and their possible refinements will be discussed with the NACWO and NVS where necessary.

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

Guidance will be obtained from published materials regarding best practice for field studies on fish populations such as the UFR committee Guidelines for the use of fishes in research (2013) American Fisheries Soc.

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

We are committed to using best practices in implementing the 3Rs to reduce potential impacts of the work on individual fish and the fish populations under study. We will stay informed of advances of the 3Rs through institutional communications, checking the NC3Rs website and following the technical and scientific literature. Advice and further up to date information will be obtained from the named persons (eg NACWO, NIO, and NVS).

**A Retrospective assessment of Refinement will be due by 19 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?

# 25. Evolution and adaption of bacterial pathogens within the host

**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**

bacteria, infection, niche adaptation, therapeutic intervention, colonisation

| 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?**

The aim of this work is to understand how bacteria evolve and adapt to specific environments found within different body sites. Such information is key if we are to understand how these bacteria colonise, persist and damage the human host. Availability of such knowledge is also essential for the design of new drugs and vaccines that interfere with these processes, prevent infection and subsequent disease.

**A Retrospective assessment of these aims will be due by 11 July 2026**

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

- Did the project achieve it's aims and if not, why not?

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

**Why is it important to undertake this work?**

Antimicrobial resistance is a global issue that threatens society by limiting our ability to prevent bacterial infections previously considered as treatable. To design alternative and effective treatments, we must understand how these organisms respond and adapt to the specific challenges found at the body sites that these organisms preferentially infect. Further, we should consider how factors such as diet and antibiotic treatment can alter the local environmental conditions, as the impact of such changes may alter the capacity of some disease-causing organisms to flourish and exchange genes involved in the spread of microbial resistance.

Laboratory studies of many medically important bacteria have revealed a huge flexibility in the capacity of these organisms to respond to environmental changes, rapidly modifying the genes they express when exposed to changes in environmental factors including pH, oxygen, temperature and nutrition. In the laboratory, the influence of these types of change can be monitored by studying the way the bacteria respond to each specific individual environmental condition. However, in the animal or human host, each site within the body offers a unique set of conditions to which the bacteria must respond, the combination of which we are currently unable to artificially reproduce in the laboratory. In contrast, the study of infection within the animal host offers an opportunity to identify those genes which are uniquely turned on in response to conditions found at the preferred site of infection that allow successful colonisation, persistence and survival of the pathogen. Recent technological advances are allowing us to measure such changes in gene expression and offers an opportunity to reveal/confirm how these disease causing organisms respond to the environmental cues found at different body sites. This information is key if we are to design new drugs and vaccines that can more effectively interfere with these processes and prevent infection.

To achieve this goal, we have developed an approach that allows us to recover and analyse the nucleic acid (messenger RNA) made by bacteria during infection of the host. Messenger RNA (mRNA) contains all the information that is needed to make new proteins as the bacteria change and adapt to the changing conditions in the host. It is relatively short lived and by capturing and analysing this information, its is possible to follow over time, adaptations the bacteria makes. This includes production of new structures that are key for attachment to the host cells and adaptations that help it survive within the niche and make best use of the available nutrients. Using this approach, we can look at the global changes the bacteria makes in response to its arrival, persistence and onward transmission from different body sites, identifying genes crucial for these processes. The importance of these genes to the infection process can then be confirmed through the testing the ability of bacteria, with specific mutations in these genes, to infect animals. If the role of the identified gene in disease is confirmed, it should provide a target to which new therapeutic interventions can be designed.

We are now able to study gene expression from bacteria recovered directly from the gut tissue of infected mice and have shown that compounds produced by commensal bacteria

found in the healthy gut can be recognised by a number of disease-causing intestinal pathogens, including Salmonella and *Escherichia coli.* These appear important cues or signals that the bacteria that they have arrived within the gut, stimulating the production of several proteins that help the bacteria stick to the gut surface. It is therefore possible that the foods we consume may be important in the survival and subsequent damage these organisms cause. We wish to extend our studies to identify those genes expressed by *E. coli* during acute and chronic infection at sites other than the intestine, including the bladder and kidney, as well as understanding the role of other genes we know are expressed during intestinal colonisation and disease.

The bacteria described in this application are all recognised to cause significant infections of humans and for which antibiotic resistance is an increasing challenge to treatment. Understanding their capacity to colonise and adapt to survival in the gut, urinary tract and bloodstream is essential for reduction of chronic urinary tract infections which can lead to sepsis, whilst knowledge of those genes involved in adaption of *C. difficile* to the antibiotic-treated gut offers to limit transmission of infection within the hospital setting.

Further, we hope to use our experience to understand the natural role of the gut in the spread of antibiotic resistance genes. In particular we wish to determine the role of bacteriophages (natural viruses of bacteria) in the transfer of antibiotic resistance genes between bacteria in the gut. This is because rates of transfer appear increased when the bacteria are stressed by factors including lack of nutrients and exposure to antibiotics. It is essential that we are able to determine now antibiotic resistance genes are transferred between bacteria within the gut, so that we can limit the circumstances that influence such events.

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

By the end of the project, we hope to have a clearer understanding of how the named bacteria adaption to, and evolve within, different anatomical sites in the body. This knowledge and information will be shared with the scientific community and beyond through the writing and publication of scientific publications, presentation of the information at national and international specialist conferences and through outreach activities to the general public. We also hope to establish whether a number of compounds previously shown to prevent these bacteria expressing adhesins and virulence traits that are responsible for causing disease.

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

What: The outputs of this project will largely benefit members of the academic community who study how the named pathogens colonise and persist within different anatomical sites of the body. As environmental conditions within these sites are difficult to mimic in the laboratory, studies that allow global analysis of gene expression should help define questions for academic study and in the longer- term provide new targets for interventional therapies. In particular, these studies will establish whether modification of environmental conditions within a particular body site (for example as a consequence of antibiotic treatment or modification of diet) contribute to enhanced infection. In the longer term, the work may benefit the NHS and patients by identifying targets to which new drugs or treatments that block the capacity of the organisms to colonise the host and cause disease; in the case of *C. difficile* potentially reducing transmission within the hospitals setting. This work may also benefit industrial partners by providing evidence of drug efficacy for two new proposed treatments of that would help secure s the money required to allow further

development of these treatments. Finally, academics and policy makers will benefit from increased understanding of the role that bacteriophage play in the spread of antibiotic resistance genes in the natural setting of the gut.

Who: In the short term: these data will be used by academic teams identify and understand the contribution of genes whose expression is dependent on site-specific environmental cues. RNAseq data generated from gene expression experiments will be shared with other academic teams, through the archiving of data on a publicly accessible platform. For *C. difficile*, data produced from the creation and testing of mutants in genes linked with colonisation will be shared with the academic community and clinical colleagues with an interest in the capacity of the organism to damage the gut and transmit between hospital patients. Finally, knowledge of the role of bacteriophage in the spread of antibiotic resistance genes will be used by academics and clinicians to help understand how fast resistance mechanisms can spread and the factors that trigger this spread within the host.

In the long term: increasing our knowledge of gene expression in response to a particular niche will undoubtedly lead to more effective strategies to treat and reduce colonisation by the named bacteria. Furthermore, the approach may help to identify biomarkers that could assist in the early diagnosis and treatment of these infections.

How: the outputs from these studies will inform other scientists working in closely related areas through presentation of the data at conferences and through peer reviewed publications. They will help to confirm or refute the role of specific genes in infection (C. difficile, ExPEC infection), establish whether therapies based on drugs and new protein antibiotics are valid and help us clarify the role of phage in antibiotic resistance transfer.

When: Identification of upregulated gene expression associated with niche adaptation of these pathogens, as well as determination of the efficacy of two new drugs should be achievable within the lifetime of this licence. Other outputs from this work are likely to provide information which in the longer term may lead to more effective treatment of these diseases.

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

We will disseminate the outcomes of our studies more widely amongst the scientific community through attendance and presentations of the data at appropriate conferences and seminars.

We will also offer support to other members of the research community within the UK, by sharing the knowledge that has been gained. In the past, this has been offered the form of direct scientific collaborations which have resulted in a number of highly productive collaborative efforts with both academic and industrial groups, or in the form of training to those interested in seeking project licences to use these models for their own work.

We have described our approaches to the study of infection in publications and will continue to combine aspects of new and developing technologies with *in vivo* models to maximise outputs.

**Species and numbers of animals expected to be used**

- Mice: 2400

# Predicted harms

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

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

Adult mice will be used in these studies as they allow us to study the dynamic and specific interactions that occur, between each of the named bacteria and the host, in real time. As features of the infection, such as colonisation parallels that observed in humans, mice offer an opportunity to understand the key steps in these processes, allowing us to identify and test drugs or vaccines that interfere or limit these interactions. Mice offer the best model as we have significant knowledge of their immune systems and we have developed a wide range of tools (including antibodies that recognise specific cells of the immune system) that can be used to understand the role of the host response in disease. Animals around 8-10 weeks of age are fully mature, have an intact immune system capable of recognising and responding to bacterial infections.

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

Typically, animals will be infected by either direct introduction of the organism into the stomach (using a feeding needle), into the bladder (via a catheter) or into the blood by injecting the bacteria into tail vein of the mouse. Colonisation of the vagina involves the direct introduction of the bacteria into the vagina.

Pre-treatment, prior to infection, with antibiotics or other interventions is sometimes required to increase susceptibility to infection. Where this is necessary, antibiotics/drugs will either be offered in either the food or drinking water or through direct treatment (via injection or feeding needle).

Infection success will largely be determined through the recovery of the infecting organism in the faeces or through the light that genetically modified bacteria have been engineered to emit. Using this approach, the growth and persistence of the bacteria within each animal can be monitored using a specialist piece of equipment capable of detecting this light. This method is known as *in vivo* imaging (IVI).

The number of procedures (injection, exposure to bacteria, anaesthesia) carried out on each animal will be carefully considered, with the minimum number of procedures employed to answer the experimental question. In the majority of cases, animals will be subject to only those procedures required to prepare, infect and follow the growth and persistence of the bacteria within the host (by collection of faecal samples or measurement of light).

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

For several of the models (intestinal colonisation with *E. coli* and *C. rodentium*; urinary tract infection of
*E. coli*; vaginal colonisation with *S. agalactiae;* intestinal colonisation with *S. aureus*) - the impact on the animals will be limited to the stress associated with the procedures used to infect the animals.

For *C. difficile* models, animals can develop diarorhea and lose weight. This can occur from 12h post infection and is transient, with most animals regaining any lost weight 3 days post infection. Animals are helped during this time by providing wet, mashed and easily accessible food on the floor of the cage. Any animal that does not regain weight amount after 2 days or loses an unacceptable amount of weight (up to 20% of its starting weight) during this time will be killed.

For intravenous (i.v.) infections with *E. coli*. In general, animals are infected for very short periods of time (up to 18h) and do not get sick. In longer experiments, it is possible that the multiplication of the bacteria could lead to the development of sepsis. To limit overwhelming infections, we will monitor weight loss as this is a good indicator of disease severity. Any animal that loses an unacceptable amount of weight (up to 20% of its starting weight) or shows a continuing weight loss over more than 3 days will be killed.

For intestinal challenge with *C. rodentium* that express the Shiga-like toxin (Stx), damage to the kidneys can be significant and can be seen in animals from 6 days post infection. Weight loss has been shown to correlate well with kidney damage and suffering will be minimised by killing any animals that show a sustained weight loss of between 6-10% over a 48h period.

**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 each animal will suffer will vary from mild to moderate depending on the method of infection and strain of bacteria used. All animals subject to infection would be expected to experience some level of moderate discomfort, although this is likely to reflect the discomfort associated with the procedure used (oral gavage, instillation of organisms into the vagina or bladder).

Animals infected with unmodified, disease-causing strains of *C. difficile*, with the toxin producing strain of *C. rodentium* (stx) or with strains of *E. coli* injected into the blood could, if not monitored appropriately, experience severe disease following infection. Careful monitoring, together with clearly defined experimental endpoints (such as loss of a specific amount of weight) will limit the suffering of any individual animal to moderate discomfort.

Analysis of genetically modified strains of the bacteria, in which virulence associated traits have been deleted, are likely to be less infectious than the unmodified strains. Consequently, they are less likely to recapitulate the full spectrum of disease, reducing further the level of discomfort experienced by these animals.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 11 July 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?**

Infection is a dynamic process in which disease outcome is often dependent on several responses and counter-responses by both the organism and the host. This interaction is further complicated by the fact that bacteria can respond to the specific environmental conditions at different body sites. While individual parts of the infection process can be studied in the laboratory (and will be undertaken as a first approach where possible), full understanding of how bacteria grow and persist in the host is difficult. This can only be determined when multiple factors including host immune responses and local environmental conditions can be considered simultaneously.

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

Several aspects of the research have already been tested using non-animal alternatives. For example, drugs designed to specifically limit the capacity of Enterohemorrhagic *E.coli* (EHEC) to adhere to cells and produce toxin, have been synthetically created and tested using cell-based systems in the laboratory. Using this approach, we have modified these drugs to enhance their solubility, eliminate their toxicity and consider how these drugs can be used in combination. The use of cultured cells will continue and should ensure that only the most efficacious, non-toxic drugs are tested *in vivo*. Similarly, the protein antibiotics that may reduce vaginal colonisation by Group B Streptococci (*S. agalactiae*) have already been studied in a variety of models including the invertebrate waxworm maggots. Only those candidates that have shown promise with respect to antimicrobial activity, structural stability, and activity against a range of *S. agalactiae* strains have been selected for further testing in the animal.

We are also continually seeking to develop new laboratory-based systems in which specific features of the infection process can be modelled. For example, we are developing an oxygen-limited, cell-based system that mirrors the environment of the large bowel; an environment in which the obligate anaerobe *C. difficile* can flourish. This should allow us to determine which bacterial proteins enhance colonisation and persistence within the niche. In addition, our increasing knowledge of bacterial adaption to the site of infection, will offer further opportunities to recreate these conditions artificially within the laboratory, potentially reducing animal use in futurestudies.

**Why were they not suitable?**

While artificial recreation of one or two conditions found within a particular body site offer insight into specific aspects of the infection process, it cannot reproduce the dynamic and ongoing process of infection. They do not consider the role of enzymatic activity as typified by host proteases, encountered during transit down the intestinal tract to break down or modify compounds that appear structurally intact *in vitro*. Further, such studies fail to reflect the impact that metabolites produced by the microbiota, or immune responses activated in response to infection, modify gene expression by these pathogens.

**A Retrospective assessment of Replacement will be due by 11 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

The number of animals estimated is based on the programme of work described and existing experience of the minimal number of animals needed in each experimental group to provide statistically robust outcomes. Group sizes are constantly being re-evaluated and updated when necessary. In situations in which new mutant strains, or modification to methods are used, work will initially be limited to small numbers of animals, with several sequential experiments undertaken. This will allow us to measure the inherent variation in outputs between experiments, which will help determine the number of animals required for subsequent experiments.

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

During the experimental design phase, the NC3Rs Experimental Design Assistant provided guidance on experimental design to include factors such as randomisation of animals and blinding of experiments. This was also used in the calculation of approximate experimental group sizes, to determine (based on existing experimental data) the minimal number of animals that should be considered for use in each experimental group.

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

Whenever possible, numbers of control animals will be minimised through the testing of multiple mutant strains of bacteria in one experiment. Techniques including competitive index analysis in which co- infections of mutant and wild type bacterial strains within one animal, will be used to determine the relative biological fitness of a mutant to compete with an unmodified strain. These experiments reduce the number of animals by avoiding the need to include control animals infected with wild type strains alone and allow direct comparison of infection in the same animal. Disease is not enhanced because of co-infection.

Furthermore, we are developing new techniques that allow us to study the relative proportion of each mutant based on unique sequences of DNA. This will allow us to evaluate several mutants in a single animal and reduce the number of animals required in future experiments.

Tissue and blood from infected animals will also be preserved where possible to generate a biobank of material, offering an opportunity for further testing without the need to use further animals.

**A Retrospective assessment of Reduction will be due by 11 July 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

The animal models described replicate several features of the infections associated with the named bacteria in humans. This includes gastrointestinal infection by *C. difficile* (acute and relapsing disease), intestinal infections by Enterohemorrhagic *E. coli* (EHEC), non-intestinal infections by strains of *E. coli* that survive and replicate in the blood and the urinary tract, and vaginal colonisation by *S. agalactiae* (known as group B Strep). They have been developed to support the study of the fundamental interactions between bacteria and the diseased host.

Most of the methods outlined in this application are subject to continued modification to limit animal suffering. Refinement in recent years, has seen movement away from animal models in which disease is potentially fatal (*C. difficile* in Syrian Hamsters) to mouse models in which study of the key features of infection is feasible but disease is limited. Similarly, we are increasingly using strains of *C. difficile* which are unable to produce the toxins that are responsible for much of the intestinal damage caused by this bacterium. These strains allow us to study features of the infection, such as colonisation and transmission, whilst reducing animal suffering.

New technologies, including the capacity to sequence recovered nucleic acid (DNA and mRNA) is helping us understand which genes are important in disease, providing opportunities to refine experiments by allowing multiple mutants of bacteria to be studied simultaneously within one animal.

Animal stress has also been reduced by the adoption of non-aversive handling techniques.

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

Study of infection is a dynamic process that requires the presence of both the pathogen and a mature immune system capable of responding to the pathogen as it grows and

adapts to local conditions within the site of infection. Adult mice are used in these studies as younger animals fail to respond as effectively to bacterial infections and rapidly become overwhelmed with disease. Similarly, whilst we have studied some of these interactions in the waxworm maggot model, these insect studies offer limited opportunities to understand the adaptations made by the bacteria to avoid clearance and counter responses made by host. In addition, infection in the animal allows us to consider gene expression during different phases of the infection process. This includes understanding the role of both the bacterium and the host during the acute phase of the disease, the role of the host response in controlling infection phase and the mechanisms associated with infection clearance. This overall picture of infection would also not be possible using terminally anaesthetised animals as this would limit the time over which these events could be studied.

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

Procedures will undergo continual review to ensure any improvements in methodologies that can further reduce the number and degree of severity suffered by animals.

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

Guidance has been sought through the NC3Rs website and use of their online Experimental Design Assistant, and through guidance highlighted by our local Animal Welfare and Ethics Review Board (AWERB) team. Regular E-mails updates about 3Rs advances are also regularly provided via the Home Office Licence Liaison Contact (HOLC) and NTCO.

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

Advances in 3Rs are continually provided via email updates, NC3Rs seminars and culture of care subgroup outputs. These provide updates on new approaches and offer guidance regarding published methods. There are also opportunities to discuss new methods with the named Veterinary Surgeon (NVS) and Named Training and Competency Officer (NTCO) who offer help and support around training for the adoption of new methods. The NC3Rs website provides an additional excellent resource.

**A Retrospective assessment of Refinement will be due by 11 July 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?

# 26. Mechanisms and treatment of pulmonary vascular 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
- 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**

Cardiovascular, Endothelium, Genetics, Therapy, Pulmonary hypertension

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

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is 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?**

This project aims to determine the mechanisms of genetic forms of a rare disease, pulmonary arterial hypertension (PAH). We will use this knowledge to develop and test new treatment strategies for pulmonary arterial hypertension and other related conditions.

**A Retrospective assessment of these aims will be due by 04 September 2026**
The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

- Did the project achieve it's aims and if not, why not?

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

**Why is it important to undertake this work?**

Pulmonary arterial hypertension is a rare disease with a relatively poor chance of survival once the disease is diagnosed. Despite the availability of existing licensed treatments, the average rate of death at 3 years after diagnosis is 40%. We aim to develop new approaches to treatment that target the pathways identified from human genetic studies. Targeting pathways identified from genetic insights are more likely to lead to successful treatments that improve survival as well as symptoms. The use of rodent models of PAH, including genetic models, provides important preclinical proof-of-concept for these new treatments.

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

Outputs from this project will include new knowledge, which will be captured in high impact publications in internationally recognised scientific journals. In addition, we will provide proof of concept for the use of new drug approaches in pulmonary arterial hypertension.

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

The major beneficiaries of these outputs will be scientists, clinicians and patients with PAH. In the short term, validation of human genetic findings in genetically modified animals provides important evidence for a causal role of mutations identified in patients with PAH. This evidence will support genetic testing and counselling advice to patients. In addition, in the longer term, drugs shown to be effective in our project can be advanced into the clinic for testing in patients with PAH.

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

We will disseminate new knowledge and findings by publishing our results in internationally recognised scientific journals. In addition, we will publicise our outputs via the communications teams of our institute and funders. We will also use social media to publicise our outputs. We will attend meetings of PAH patient groups and disseminate our research findings in their newsletters. We collaborate widely with international experts, when necessary. This is particularly important when working on a new gene or pathway that might be unfamiliar to us. We collaborate with commercial partners when possible to hasten the translation of our findings into the clinic for the benefit of patients. Whenever possible we will publish and disseminate any negative research findings, for example via the Faculty of 1000 Open Research Platform.

**Species and numbers of animals expected to be used**

- Mice: 6000
- Rats: 900

## Predicted harms

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

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

We will use adult rats and mice for the majority of our proposed studies. Typically, mice carrying human PAH disease-causing mutations only develop minor changes in their lung blood vessels at 3 months of age. We have found that mice at 6 months of age, or with some mutations 1 year, develop an age- related pulmonary hypertensive phenotype. Mice remain our preferred species for assessing the impact of disease-causing mutations on the disease-associated changes in the structures and responses of the lung blood vessels and heart, and we have adapted our invasive and imaging protocols for this species. For the study of non-genetic forms of PAH, the rat is a more robust model than the mouse. For example, the Sugen-hypoxia protocol in rats, representing the combination of a drug (Sugen) and low oxygen levels similar to those experienced by people at a high altitude (hypoxia), leads to the development of marked and sustained PAH and disease-associated changes in the lung blood vessels that, of existing rodent models, most closely resembles human PAH. Mice do not respond to the same extent and often do not develop thickening of the inside surface of the lung blood vessel wall to the same extent as is seen in rats and humans. Therefore, the use of rats and mice is necessary and complementary to the objectives of this project.

In a small number of experiments, we may need to use young mice (5-8 weeks of age) that have already been weaned. This will happen with mice carrying gene mutations that only appear when a drug, such as Tamoxifen, is injected. This will be carried out in cases when we need smaller mice to test the effects of expensive drugs, or if we need to isolate lung cells, which grow more successfully if isolated from mice between 5-7 weeks of age.

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

Injections and procedures will be kept to a minimum to achieve the required results of the experiments. Pilot and pharmacokinetic studies will be performed on new molecules and drug candidates in order to determine dose levels and dosing frequencies required to assess efficacy. The most appropriate route of administrations will be determined by pilot and pharmacokinetic studies or taken from appropriate published literature.

Animals will be kept under anaesthesia for the minimum time possible to achieve the required results. Typically, the catheterisation procedure takes approximately 30-40 minutes to complete, depending on the severity of disease. While under anaesthetic animals will be maintained at an appropriate temperature by the use of a heat mat or similar heating device. Following aseptic recovery surgery animals will be kept in a pre-warmed recovery environment until fully recovered and freely moving.

Chronic hypoxia studies require the animal to be at a minimum oxygen concentration of 10% for at least 21 days to achieve the desired phenotypic response and for rats to develop the excessive growth of cells at the inside surface of the pulmonary blood vessel wall that narrows the vessel. Longer durations of hypoxic exposure up to 28 days may be required in some mouse strains. Following hypoxic exposure rats will be dosed for 6-8 weeks to determine efficacy of the molecule being tested. During the dosing period animals may undergo imaging in order to track disease progression.

Substances injected will be of known safe dose concentrations and administration routes. The administration of substances 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. Small molecule inhibitors and biologic reagents will be solubilised in solutions that have been tested and deemed safe.

Blood sampling via superficial veins (usually from the hind leg) will be kept to a minimum and safe volumes calculated based on estimated circulating volumes. Pharmacokinetic studies will typically require a single dose of a molecule or substance followed by a number of scheduled bleeds. The limits for the volume and frequency of blood sampling from laboratory animals, as defined according to Wolfensohn & Lloyd, 2003, Handbook of Laboratory Animal Management and Welfare, 3rd Edition and the National Centre for the Replacement, Refinement and Reduction of Animals in Research website, will be adhered to.

In a small number of animals, we will undertake pulmonary artery banding, where a small loop is tied around the pulmonary artery, between the right side of the heart and the lung, to reduce the flexibility of the vessel wall and place some pressure on the right heart. In patients with genetic mutations, it has been observed that their right hearts increase in size, but do not become more muscular, so cannot cope with the increases pressures that are found in PAH patients. This leads to a more rapid failure of the right heart. In other models, where the lung blood vessels are also involved in the disease process, we cannot specifically study the changes in the right heart, or it's response to potential treatments. The pulmonary artery banding allows us to explore the response and effects of treatments on the right heart directly.

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

Genetically altered mice produced under this protocol are not expected to exhibit any harmful phenotype. Some animals will experience mild to moderate transient pain associated with an injection or blood sampling procedure.

From our previous experience, a subset of rats that are exposed to the PAH-inducing stimulus of a single injection of Sugen followed by 3-weeks hypoxia (Sugen-hypoxia) with subsequent maintenance in normal air during which a progressive pulmonary hypertensive phenotype develops may experience right heart failure. Right heart failure occurs rapidly without any obvious phenotypic symptoms. We have refined this model to minimise the number of animals experiencing this response.

In the pulmonary artery banding model it is anticipated that a small proportion of mice (<10%) will die under anaesthesia, during recovery from anaesthesia or within 2-3 weeks of surgery. Deaths under anaesthesia or during recovery from anaesthesia usually occur if the banding of the artery leads to very high pressures in the right ventricle leading to acute right heart failure. This will be apparent based on clinical signs during recovery from surgery and the animals will be killed by a Schedule 1 method if this is observed. Death in the period of 2-3 weeks after surgery may be due to the development of pericardial effusion. Animals will be monitored for evidence of clinical signs and will be killed by a Schedule 1 method if this is observed.

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

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

Overall, the expected severities in mouse studies will be mild. Some models may exhibit a moderate phenotype, but this is anticipated to be less than 5% of all mouse models. Mice on the PA banding protocol might experience a severe phenotype due to the nature of the surgery, though this is expected to be less than 1% of all animals.

Overall, the expected severities in rat studies will be moderate, primarily as the Sugen-hypoxia model is the model of choice with regard to relevance and refinement. The proportion of rats anticipated to experience a moderate phenotype is 25%.

**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 04 September 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

Although we routinely use human cells and tissues, as well as molecular and biochemical approaches to achieve our research objectives, the biology of these systems is restricted compared to the complex compositions of the cell types involved in cardiac and vascular remodelling in the whole organism. For example, bone marrow-derived cells may contribute to the process of pulmonary vascular remodelling or protect against remodelling. It is not possible to incorporate the complexity of the animal situation into these cell based models. In addition, different regions of the lung may influence each other, such that there may be influences from the alveoli or lymphatic system on pulmonary vascular remodelling, and complex interactions between cell types in the vascular wall. Moreover, distant organs influence the lung vasculature via factors circulating in the blood. For example, changes in liver function influence the function of the pulmonary circulation, as evidenced by the occurrence of PAH in patients with liver disease.

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

We would emphasise that our animal studies are greatly informed by large scale human genetics studies in patients in PAH. Our animal research is conducted only when we have a very high degree of certainty of the importance and impact of the research based on

observations from human genetics. We strive to use human-derived material wherever possible to undertake mechanistic studies and to screen for drug effects. For example, we use human stem cells in which we introduce mutations in specific genes to examine the effect of PAH causing mutations on cell function. In addition, we have developed techniques to isolate blood outgrowth endothelial cells from patients and controls to undertake studies of specific gene mutations. We employ extensive cell-based approaches to validate the roles of specific genes or pathways prior to embarking on animal studies. Thus, the cell- and animal-based approaches are used in a complementary manner to achieve our research objectives, but animal studies are only embarked on once we have reason to believe that a particular gene or pathway is likely to be central to a disease and could be tackled by using suitable drugs in patients.

**Why were they not suitable?**

It is not that non-animal alternatives are not suitable; it is rather that in isolation they are not sufficient to achieve our objectives. The combination of non-animal and animal approaches is required to generate the confidence to move forwards into clinical studies in humans and patients with PAH.

**A Retrospective assessment of Replacement will be due by 04 September 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

The number of animals has been estimated based on our extensive experience over the past 15 years with animal (mouse and rat) models of PAH, allowing for the improvements in experimental design and refinement of our protocols that have taken place over that time. For example, we know approximately how many mice it takes to breed a colony ready to undertake a full phenotyping assessment. In addition, we know how many mice or rats are needed to address a specific question to achieve our objectives. We have assumed that each gene or pathway (we anticipate investigating 4-8 such pathways) under investigation will be pursued to conclusion in estimating these numbers. In reality, we may terminate a programme of work early if the emerging results do not justify continuation. This is kept under continuous review.

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

We use historical research data from our laboratory databases to appropriately power our studies depending on the desired endpoint. The endpoint might be measurement of blood

pressure within the lungs, for example to determine whether a genetically altered mouse has a significantly higher pressure than wild type littermates. Alternatively, the endpoint might be measurements of the degree of thickening of the walls of blood vessels within the lung, or the response to a drug intervention. In the latter case we decide beforehand what represents a meaningful reduction in pressure or increase in heart function and power the experiments based on this.

We have significantly reduced the numbers of animals used in our phenotyping studies by using ultrasound to track the progression of pulmonary hypertension longitudinally in genetically altered mice. This means we can study the heart function of the same mouse twice by ultrasound at two different time points. With new genetic mouse models, we do not know at what time point animals develop PAH. In the past we would have needed to undertake terminal catheterisation of the right side of the heart in two groups of animals at, for example, 3 and 6 months to determine this. With ultrasound we can track pressures over time and can use half as many animals. For example, in a recent study in genetically altered mice we measured heart function by ultrasound at 4 month, 8 months and 11.5 months of age. In this study we used 45 mice, as opposed to the 135 mice that would have been required for pressure monitoring by catheterisation at each point. This approach has more than halved the required animal numbers in these protocols.

Where appropriate we will use the web-based Experimental Design Assistant available from the National Centre for Replacement, Refinement and Reduction of Animals in Research to ensure that we use the minimum number of animals consistent with our scientific objectives and undertake appropriate statistical analysis. We will also follow the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines for planning animal research and testing.

We will follow the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) during the conduct of our experiments to support publication of data of maximal quality, reliability and reproducibility.

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

Mating will be set up according to the requirements for specific experimental designs to reduce the risk of overproduction of animals. Mice will be genotyped rapidly to permit early assignment to experimental protocols and enable the separation of breeding pairs once numbers that are sufficient to achieved power have been reached. Multiple organs will be harvested to allow for future analyses that may not be relevant to the specific experimental aim. Tissue and tissue extracts will be stored long-term to enable future analyses for new targets or sharing with other researchers.

**A Retrospective assessment of Reduction will be due by 04 September 2026**

The PPL holder will be required to disclose:

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

## Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative**

**care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.**

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

We will use mice carrying changes in their genes that are similar to the human disease. The human disease develops slowly and only affects about one in five people who have the damaged gene. We believe that the effect of the gene damage is often only revealed if something else happens in the lung, such as an infection or exposure to pollutants. We may need to expose mice with the damaged genes to a factor that increases their chance of developing disease. The disease that develops in the lung blood vessels does not cause any obvious signs that these animals are developing disease.

There are no robust rat models with damage in the disease relevant genes, so we have to give them a drug and reduce their oxygen levels (similar to being on the top of a mountain for a few weeks) to start the disease process. Once this is started, the disease gradually worsens. We have changed the method to reduce the disease variability between rats and to make the process shorted by 4 weeks, so that the length of the total process until we test the level of disease is 7 weeks. During this period, the rats do not show any obvious external signs that they have disease, but their lungs will have developed disease. However, the amount of lung disease that is present in these rats is enough that we can test compounds that may represent future drugs for use in humans with the disease.

The major determinant of survival in humans with PAH is the response of the right ventricle to the increased pulmonary artery pressure. It is failure of the right ventricle that leads to early death. In mice, the available stimuli for the development of pulmonary hypertension result in adaptive hypertrophy of the right ventricle but not in right heart failure. In order to determine mechanisms and potential interventions for right heart failure, a model is employed that involves partially ligating the main pulmonary artery as it emerges from the heart. This causes a narrowing of the main pulmonary artery and greatly increases the pressure within the right ventricle, leading to right ventricular dilatation and failure. More than 90% of mice recover from and tolerate this procedure, whilst 10% die during the anaesthetic, immediately after during recovery or within 2-3 weeks of surgery as a consequence of pericardial effusion. This provides an opportunity to assess the role of specific genes and therapies that might benefit patents with PAH and right ventricular failure.

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

We cannot use younger mice for our studies as the pressure catheters we use are too large to insert into the blood vessels of younger mice. Rodents are the species of choice, because the cardiovascular systems of non-mammals do not have the same structure or responses. The disease we are examining takes time to develop, so shorter time points do not give a difference that is much different to non- diseased animals. Some of our protocols are non-recovery protocols; that is animals will undergo terminal procedures under a general anaesthetic and thus will not be sentient of the procedure.

**How will you refine the procedures you're using to minimise the welfare costs**

**(harms) for the animals?**

We will always strive to minimise any potential suffering or harms caused to the animals by ensuring that our researchers are well-trained with good communication skills; researchers will be provided with relevant literature to ensure awareness of the best practices; staff will undertake training courses to ensure their skills are updated and current and will be appropriately assessed. All researchers will adhere to the principles of refinement in accordance with NC3Rs.

For bleeding from superficial veins, animals will be placed in warming cabinets to ensure that vessels are dilated and therefore reduce bleeding time and stress.

In cases where recovery surgery is undertaken, animals will be maintained in warming cabinets and their recovery observed for any signs of post-operative distress. Appropriate pain relief will be provided.

Animals will be observed on a daily basis with the assistance of the technical staff in the unit and researches alerted to any problems as soon as possible to ensure that any health issues are dealt with appropriately, either through consultation with the NVS or schedule 1 killing according to the health issue in question.

Animal weights will be recorded and entered into the electronic MCMS database; this will be set to alert users to any weight changes that are defined according to humane endpoints.

In protocols of moderate or severe severity, scoring and monitoring methods will be implemented to ensure that animals do not exceed the adverse effects stated.

Where animals are not housed in their home cages, for example when they are housed in low oxygen chambers, the most appropriate environmental enrichment will be used. Staff will abide by the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery and pain management will be controlled by both before and after operation pain medication with the most appropriate dose for the species used.

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

The following general published guidance will be followed:

ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines version 2.0 (https://arriveguidelines.org/), published by NC3Rs to improve the reporting of research involving animals.

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery, 2nd Edition 2017

The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines for planning animal research and testing.

Specific published recommendations for experiments in animals in the field of pulmonary hypertension will be designated as required reading for researchers conducting experiments under this licence:

Standards and Methodological Rigor in Pulmonary Arterial Hypertension Preclinical and Translational Research. Provencher S, Archer SL, Ramirez FD, Hibbert B, Paulin R, Boucherat O, Lacasse Y, Bonnet S. Circ Res. 2018 Mar 30;122(7):1021-1032. doi: 10.1161/CIRCRESAHA.117.312579.

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

We receive regular updates from NC3Rs, the EU NC3Rs ECVAM information network (including Norecopa, the Norwegian 3Rs centre.) and LASA, and alerts relating to new publications and studies. We also receive information from our institute and the named persons in our institute (for example the Named Vet), and our wider research community from scientists in the same field of research.

**A Retrospective assessment of Refinement will be due by 04 September 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?

# 27. Efficacy Studies Using Rodent Models of Autoimmune 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
- 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**

Autoimmune, Multiple Sclerosis, Psoriasis, Vitiligo

| Animal types | Life stages |
|---|---|
| Mice | adult |
| Rats | adult |

## Retrospective assessment

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

**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 provide service to the clients and develop experimental models for the treatment of autoimmune diseases

**A Retrospective assessment of these aims will be due by 14 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 it's aims and if not, why not?

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

**Why is it important to undertake this work?**

We will provide a service for small companies that do not have in vivo research facilities or that do not have the specialisation or experience to carry out the work required. Such companies would find it incredibly difficult to develop a drug without evidence of efficacy in animal models. So called virtual companies rely on CRO's to provide them with preclinical data to develop their drugs. In addition, we will also provide services to pharmaceutical companies who rely increasingly on CRO's to increase their pre-clinical in vivo output. Without these services available within small specialised CRO's, new drugs to treat unmet medical needs would not be advanced to the clinic. As a CRO company we develop best possible models to undertake this work to provide service to the client and also to develop new treatment for the above mentioned diseases in house.

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

As we use animal models for the investigation of efficacy of the compounds for the treatment of diseases, we would obtain various data on efficacy, safety use of the compounds and the relevant knowledge regards to the compound in these disease models. This would also help further to develop analogues in the area of compound progression and helps to take these compounds to be tested clinically. The data would be useful for either scientific publications or for patent applications.

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

The eventual benefit from these outputs will be to the patients. According to MS society, 2.3million people are affected globally and 200 new cases are diagnosed each week in US alone only. Psoriasis affects approximately 25 million people in North American and Europe alone. It has been reported that RA affects approximately 1% of the population worldwide, making it the most common form of inflammatory arthritis. Vitiligo is a common disorder, affecting between 0.5% and 1% of the population worldwide. There is a need to find a treatment to cure the diseases and this project would help to find the compound to get into clinical development for the clients and also provide necessary scientific data information for the compound.

This project would help to identify the substances at an early stage that have unacceptable side effects.

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

The protocols which are followed would be an efficient to maximise the outputs of this work for the clients. The data generated from the works will be published after consulting with the clients. Where necessary, we will collaborate with other academic institutions, companies for the successful completion of the works.

In case of an unsuccessful outcome from the work, then this would be reported and would not be used in the future work to avoid unnecessary time waste, costs and welfare of animals. Alternative approach will be used to maximise the outputs of the work.

**Species and numbers of animals expected to be used**

- Mice: 4000
- Rats: 1000

## Predicted harms

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

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

In all protocols adult mice is widely used as it shows similar physiological response to human. To further validate the experimental data, adult rats are used where it could show clinical signs similar to human.

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

Animals will be exposed or treated with non-lethal doses of substances to elicit response in relevant disease models. The drugs and stimulants will be administered through various routes and dose regimen will be selected based on published research articles and client needs. The experiments would not last more than 12 weeks in any of the protocol. If any animal will be seen to be unwell, they will be removed after watching for minimum period of time during a day. At the end of the procedures, the animals will be humane killed. In order to avoid further suffering during the procedures, animals will be humanely killed.

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

In our experiment protocols, it is expected that the animals will not experience more than moderate except in the EAE model where mice will experience greater than moderate severity. All the animals will be monitored on daily basis and the adverse effects will be controlled with special care measures and endpoints. Animals which undergoes surgery may experience pain but this will be controlled by providing pain relief before, during and after surgery. No major problems will be associated with disease induction however allergic reactions may occur at the site of adjuvant injection. This could be reduced by injecting in the flank.

The disease interferes with the normal responses of animals and results in immobility and considerable weight loss in the EAE induced model and this effect would last for few days and tend to recover later. This weight loss up to 35% is a feature of the disease process and occurs even when animals are given food and fluid orally by gavage. Paralysed animals should have access to fresh soaked diet placed on the cage floor, which consists of soft bedding. Food and water will be provided as normal as well. Due to the presence of white scaling on the skin, no erythema increase normally observed in psoriasis models.

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

**species.**

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

In most of the specified disease models the animals would experience moderate and greater than moderate severities and this would be observed in disease induced animal models. The doses which would be used, would less likely to cause adverse effects in the animals.

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

- Killed

**A Retrospective assessment of these Predicted harms will be due by 14 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?**

During preliminary research it became preferable to use animals rather than humans as the principles of anatomy and physiology are of animals were similar to humans as the certain strains of animals get the same diseases as human therefore the animal models are used in this project to understanding the diseases and developing appropriate treatments for the diseases at controlled environment and also free from other contaminations.

Using animals in this project would help to obtain data which are scientifically valid before proceeding into next stage in humans and scientifically valid numbers are important in this aspect which could be easily managed with animals. The animals are selected based on various scientific research publications and this would help to reduce the use of non-suitable strains in the project.

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

We have considered to use in vitro technique and ex vivo technique to replicate or produce the results which can be obtained by in vivo using animals however it is difficult to find an alternative method to investigate the mechanism of inflammation in each of the specified diseases. The replacement technique would be further developed to reduce the use of the animals during the project.

**Why were they not suitable?**

In vitro techniques do provide basic information required however this do not offer the complexity of studying and modifying an disease caused by the immune system that causes nerve damage to the nervous system and other signalling pathway in each specified diseases in the project.

**A Retrospective assessment of Replacement will be due by 14 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?**

In this project, we mostly use mice to start with addressing the issues and client needs therefore the requirement of mice will be more over the 5 years when compared to other animals. Where appropriate we use rat to validate the scientific data. The numbers are estimated based on power calculations.

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

Based on Experimental Design Assistant various steps were taken such as variability, use of different strains to obtain reproducible data to utilise the animal to its full potential and use of appropriate statistics was looked at to reduce the number of animals used in the experiment. The proven experimental design which are previously published are followed to minimise any errors associated with research.

By accounting for the influence of variables and addressing sources of bias, an adequately designed experiment will yield robust and reproducible data, ensuring that the data from every animal is utilised to its full potential.

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

Use of right strain would help to minimise the variability therefore reduced number of animals could be applied. Instead of undertaking whole experiment at first instance, run pilot studies to ensure that the phenomenon could be observed. To minimise the variability, we will use animals with a defined genetic background. Randomization procedures would be used to avoid bias. Use same animal to obtain various data using histological and immunological assays therefore we obtain multiple readouts from the same animal as specified in the protocol and this significantly reduces the total number of animals that would otherwise be used.

**A Retrospective assessment of Reduction will be due by 14 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 use mice as a choice of animal in all our studies as its immune system is comparable to human and small animals are easy to handle for the initial stages of any investigation. Where necessary, rats will be used to obtain further information on diseases.

Most of the biological readouts will be undertaken under terminal anesthesia. The dose which is used to elicit or treat any diseases would be kept optimum in such a way that those animals suffering would be minimised and when surgery is required for the collection of blood or removal of organs then strict surgical aseptic and use of preoperative analgesia will be the norm. In the case when animals are treated with novel substances, and when no information is available concerning toxicity, then we will begin with a low dosing strategy to minimise animal suffering.

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

The animals are used to mimic the human physiological condition for the treatment of diseases. All our protocols are designed to use minimum number of animals and minimise any welfare costs to the animals, we use animals which are relevant to the development of pharmaceutical drugs and designed studies that address unmet clinical needs. The experiment tools we generate would help to investigate and provide the solution to the issue which would help to create human benefit.

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

All the procedures are vigorously followed to minimise the sufferings in the animals such monitoring them at regular intervals and give analgesic treatment in the case of any surgical procedures. As the protocols are designed to use minimum number of animals this would reduce the welfare costs in the animals.

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

The best practice guidance produced by the National Centre for the Replacement,

Refinement and Reduction of Animals in Research (NC3Rs) clarifies the responsibility in the use of animals in bioscience research in the most refined way. The guidance sets out some general principles for good practice on the use of animals in scientific procedures including experimental design and statistical analysis, species selection, genetic alteration, dosing and sampling, anaesthesia, analgesia, welfare assessment, humane endpoints, and euthanasia.

Identifying the animal by the ringing, tagging or marking of an animal, should not cause more than momentary pain or distress and no lasting harm and trained people should undertake humane killing of animals under schedule 1 as guided in ASPA. The administration of any substance or article to an animal should be carried out for research purposes as certified by the Veterinary Medicines Regulations 2011

http://www.procedureswithcare.org.uk provides guidance on dosing mice and rats. http://www.nc3rs. org.uk/our-resources/blood-sampling provides guidance on blood sampling in common laboratory animal species. Handling, restraint and training of animals could be learned using http://www.nc3rs.org.uk/ handling-and-restraint and http://www.nc3rs.org. uk/training-animals.

During surgical procedures aseptic technique should always be used to reduce the risk of post-surgical wound infection and the guidance on this could be obtained using http://www. procedureswithcare.org.uk and the LASA guiding principles.

The experiments should be conducted on animals which most likely to produce satisfactory results with the least degree of harm and also use the guidance to minimise the number of animals by careful planning and use of appropriate and efficient experimental designs, statistical analyses, the significance threshold and power level, the population variance and the factors and scientific significance of response.

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

During the project, if any scientifically advanced methods are available to address the any of the specified diseases through various research publications, we would implement it to reduce the use of animals in the project. Regular updating on NC3R's website and other resources such as from academics and pharmaceutical companies would help to get any advances in 3R's immediately.

**A Retrospective assessment of Refinement will be due by 14 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?

# 28. Mechanisms of Progressive Cardiac Dysfunction

**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**

Heart failure, Heart metabolism, Hypertrophy, Cardiac remodelling, Therapy

| Animal types | Life stages |
|---|---|
| Mice | neonate, juvenile, adult, pregnant |
| Rats | juvenile, adult |

## Retrospective assessment

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

**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?**

This project aims to identify key mechanisms involved in the development of different types of heart failure so as to identify novel targets for new therapies.

**A Retrospective assessment of these aims will be due by 14 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 it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be

advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

**Why is it important to undertake this work?**

Chronic heart failure (CHF) is a major cause of illness and death and affects up to 2% of the adult population in western countries. It develops when the heart is unable to provide an adequate blood flow to meet the body's demands, triggering a condition that involves impaired function of the heart, blood vessels, kidneys, lungs and skeletal muscle. Patients with CHF have breathlessness on effort, swelling of the legs, repeated hospital admissions and a high death rate. Heart failure is caused by many different conditions that increase the workload of the heart, such as high pressure due to aortic stenosis or hypertension, high volume due to leaking heart valves, loss of heart muscle due to heart attacks, or diabetes. The response to these different stresses that cause CHF varies and may require different treatments. For example, high pressure leads to more thickening of the chambers (concentric hypertrophy) whereas high volumes lead to more dilatation (eccentric hypertrophy); long-term outcomes are typically worse for pressure than volume overload. Hypertension causes slower development of CHF, with marked scarring (fibrosis), but still increases the age and risk factor-adjusted hazard of CHF by 2 to 3-fold. Different types of hypertension have different effects. The sub-type of salt-sensitive hypertension is especially likely to cause CHF with fibrosis and problems of the heart filling with blood rather than contracting (termed Heart Failure with Preserved Ejection Fraction or HFpEF). Diabetes induces CHF through complex mechanisms that involve altered heart metabolism and increases the risk of CHF by 2–8 fold. About 19% of CHF patients have diabetes. Once clinically manifest, CHF carries an unacceptably high mortality rate which is observed for all causes of CHF, despite recent innovations in therapy such as beta-blockers, angiotensin-converting enzyme inhibitors and biventricular pacemakers. Defining the different mechanisms that underpin different aetiologies of CHF is essential in order to develop more effective therapies.

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

This work is expected to significantly increase biological and disease-related knowledge and to be highly relevant to the treatment of ill health. The project should substantially increase our understanding of the mechanisms involved in cardiac adaptation to different disease stresses and the progression to heart failure, particularly redox signalling pathways that influence multiple processes in the remodelling heart. We aim to identify specific drivers of adaptive versus maladaptive cardiac remodelling, as well as pathways that promote reverse remodelling, which can form new therapeutic targets.

By elucidating underlying mechanisms and by undertaking initial experimental studies in vivo, this research programme may provide the basis for devising novel therapeutic strategies for human cardiac disease. This is vital to achieve in the longer term because heart failure imposes a major disease burden on a significant section of the adult population and leads to very substantial costs for the health service both in the UK and worldwide. Furthermore, there is a compelling need to identify more effective treatments for this condition.

Outputs will include publication in open-access peer reviewed journals, poster and oral presentations at conferences, and novel data that forms the basis for development of new treatments.

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

Results obtained in this project will be published in open-access journals continuously through the 5- year period and will therefore add to the body of publically available knowledge for the wider scientific and clinical community. Findings will also be disseminated to the scientific and medical community by presentations at seminars and conferences. They will be of value to other research groups working in the field of heart failure, including groups developing new therapies.

A significant focus of the project is to identify new therapeutic targets and potential therapies. We therefore expect that in the longer-term this work will benefit patients with heart failure and have wider societal impact by reducing the consequences of this debilitating condition.

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

All publications will be open access. Both positive and negative findings will be published. ARRIVE guidelines will be followed for publications to maximize in vivo information in research articles. New findings will be disseminated at national and international conferences and seminars.

Our group has extensive national and international collaborations which will further enhance dissemination. We also host visits from other researchers for them to obtain direct exposure to our work.

**Species and numbers of animals expected to be used**

- Mice: 40,000
- Rats: 2,500

## Predicted harms

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

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

The study will be performed using mice and rats for several reasons. Information on the role of new signalling pathways involved in heart failure that is obtained in rodents is generally translatable to the human disease because of conservation of key pathways in mammals. Methods to induce experimental models of human disease are available and established in these species as are state-of-the-art methods to quantify the features and severity of heart failure in a similar manner to human patients.

Genetic alterations that allow the study of specific biochemical pathways in the animal and the role of such pathways in disease are readily feasible. The majority of work will be undertaken using young adult mice but a small proportion of animals will be allowed to age up to 1 year in order to observe longer term effects that may occur without surgical or pharmacological induction of stress.

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

Typically animals in this project will undergo procedures that predispose to heart failure, either by surgery (for example to narrow a blood vessel) or by drugs (for example to raise blood pressure). In some animals, we will instead assess the heart's response to regular exercise. Genetically altered animals will be used to investigate the effects of specific proteins of interest. Cardiovascular function will be serially investigated (non-invasively) and substances may be given to elucidate the mechanisms involved. Additional analyses of tissues will be performed after humanely killing the animal at the end of a protocol. Typically, animals are followed up for a maximum of 3 months although a small percentage that have not undergone surgery or drug interventions will be monitored for up to 1 year. The number of procedures will be kept to the minimum necessary to pursue the main objectives.

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

Expected impacts are the gradual development of chronic heart failure. In the majority of protocols, the period of analysis will involve stages of cardiac remodelling that occur before the development of significant clinical signs of heart failure (such as impaired breathing and reduction in mobility). Any animals that do develop such signs will be promptly managed as outlined under the individual protocols or will be humanely culled as appropriate so that the duration of such effects is expected to be less than 1 day. There may be a small proportion of early peri-operative complications that will either be successfully and quickly resolved or require early culling, so that the duration is expected to be less than 1 day: please also see the response to the next question.

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

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

Animals in protocols with no surgery will experience a mild severity. The majority of animals in protocols with surgery will have a moderate severity, as factors such as pain relief and good aseptic technique will mitigate against a severe severity. However, a small percentage of animals that undergo surgery to create conditions that predispose to heart failure may die suddenly (in much the same way as may happen with people with heart failure) and be considered in the severe category. Animals that develop such early complications will be humanely culled.

**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 14 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?**

Because Chronic Heart Failure is a complex disorder involving many organs in the body, there is no feasible alternative to the use of animal models. Cardiac remodelling and heart failure are complex chronically developing conditions involving interactions among multiple cell types and organs and characterised by changes in blood pressure, cardiac output, tissue perfusion, metabolism, inflammation, matrix remodelling, energetics and cell death. There is no suitable alternative to animal models for studying this complex and chronically developing condition.

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

Computational modelling and cell culture studies

**Why were they not suitable?**

These models are unable to fully recapitulate the integration and interrelationship between different cell types in the heart, between the heart and the vasculature, and between the cardiovascular and other body systems - all of which are important in chronic heart failure. This integrated picture which directly impacts on the clinical presentation of the condition cannot be studied in cell culture studies nor is it amenable to computational modelling due to the numerous uncertainties/unknowns regarding interlinked mechanisms. However, we will complement animal studies with cell culture studies (including heart cells derived from human stem cells) to further study specific mechanisms in greater detail, once identified from the animal experiments.

**A Retrospective assessment of Replacement will be due by 14 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 experimental design and analysis methods are based on careful consideration of statistics, power analyses and good laboratory practice and have undergone stringent review as part of the grant- awarding process. Individual experiments generally involve

factorial design to maximise the information obtained from the minimum resource. The majority of measures are quantitative and suitable for statistical analysis. Comparison between groups will be made by 1-way analysis of variance (ANOVA) or 2-way repeated measures ANOVA followed by appropriate post hoc testing. The exact numbers of animals required will vary with specific experiments and the estimates of coefficient of variation for specific outcome measures but will follow this general principle. Total numbers are also based on breeding considerations for gene-modified animals. For qualitative experiments (e.g. immunohistochemistry), the amount of material required will be the minimum necessary to provide an adequate description.

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

The experimental design implements repeat measures (for example advanced non-invasive ultrasound assessments) that are taken “in life” in each animal to track disease progression without killing the animal, which significantly reduces the total numbers required. Repeated assessments also reduce experimental variability by allowing comparisons at different time points in the same animal. At the end of the experimental period, when animals are humanely killed, we have developed efficient protocols that ensure the maximum possible experimental data are obtained using tissues. This also significantly reduces the total numbers of animals required.

We will continue to take advantage of the experimental design tools to keep numbers low e.g. NC3Rs. Online advice resource portal. https://www.nc3rs.org.uk/topic-specific-resources-0

Sample sizes for most quantitative experiments will be set by power analysis using a significance level of 5% and a power ≥80%. For example, in a four-group experiment where a difference between groups of at least 25% needs to be detected, if the coefficient of variation is 15% then about 8 animals/group would be required.

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

Principles of good experimental design will be followed to ensure clear answers to questions being addressed while using the minimum number of animals. For many studies, non-invasive techniques that allow serial assessment of cardiac function will be used, allowing reduction in numbers. This is especially valuable when assessing the impact of medicines aimed at preventing or slowing CHF. Where possible, additional information will be obtained from studies in cultured cells. For gene-altered animals, where suitable lines already exist (established by searching databases), animals will be obtained from the relevant supplier rather than breeding the animals ourselves.

**A Retrospective assessment of Reduction will be due by 14 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.**

The study will be performed using mice and rats because the experimental models mimic human disease and all relevant methods and techniques are successfully established in these species. Genetic alterations are readily available in these animals and allow the study of specific biochemical pathways in the animal with a view to understanding their role in the disease, and manipulating them to provide new treatments.

Mice will mainly be used. The major advantage of using mice is the wide availability of genetically altered lines or the ease of their generation, allowing the impact of specific genes to be examined far more specifically than achievable with most pharmacological tools. Despite their small size, state-of- the-art techniques (e.g. high-frequency echocardiography, MRI, telemetry) have been implemented to assess rodent cardiovascular structure and function in an analogous manner to humans. Models of cardiac remodelling and CHF in rodents are well established and characterised. There are some recognised differences between rodents and humans (e.g. in the heart rate and rate of progression of cardiac disease). Nevertheless, rodents have proven to be a very useful species in which to undertake cardiovascular studies (complemented by work in tissues and in human settings where feasible) and there are striking examples of new therapies that have resulted from initial studies in rodents – e.g. angiotensin-converting enzyme (ACE) inhibitors. Rats will be used less frequently; their larger size may make them a more suitable model for some studies involving gene transfer or in cases where experimental techniques/reagents are better established in this species.

Choice of models and methods:

The models to be used to induce cardiac remodelling mimic the major causes of human CHF and are all well established and validated in the published literature.

Aortic constriction is the most widely used model to induce pressure overload. A refinement we have introduced for thoracic constriction is the use of a minimally invasive procedure without open-chest surgery, which reduces mortality.

The aortocaval shunt model is a very reliable model for induction of volume overload in small animals.

Agonist (drug)-induced remodelling. The models chosen mimic renin-angiotensin system driven hypertension and salt-sensitive mineralocorticoid-dependent hypertension.

Induction of diabetes. Streptozotocin-induced diabetes is the most widely used model of type I diabetes. We will use doses and protocols validated in our laboratory as inducing diabetes of the required severity without causing severe illness or death. For type 2 pre-diabetes and diabetes, we have chosen altered diet as the most physiological method, in preference to genetic models such as the ob/ob mouse which are difficult to breed. The combination of salt-sensitive hypertension with altered diet is a very reliable murine model of HFpEF, a type of human CHF that is common but has no evidence-based therapies at

present.

Aortic deconstriction. This is the only model of reverse remodelling in mice that is reasonably well established.

The methods to be used to obtain experimental measures in living animals are the most refined available for the assessment of cardiac structure and function in rodents. We will use state-of-the-art echocardiography and imaging methods, including some that we have developed ourselves and published. Haemodynamic assessment performed as a terminal procedure again uses "gold-standard" pressure-volume analysis methodology.

All surgical procedures under all Protocols will be conducted under aseptic conditions, with appropriate pain relief, the highest levels of post-operative care and appropriate veterinary consultation. In the first 24 hours after surgery, animals will be closely monitored at frequent intervals during this period.
Animals will be reviewed at the end of the working day on the day of surgery and any considered likely to die overnight will be humanely killed. Careful attention will be paid to heating, pain relief, body weight loss, surgical wound-sites, hydration, and signs of pain or distress. During the chronic progression to CHF in all Protocols, animals will continue to be carefully monitored and any that are in a poor clinical condition will be humanely killed within 24 hours if there is no improvement.

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

We need to use mammals which closely represent the human condition and that are representative of the complex interactions that occur between body systems.

Small rodents will be used, mainly mice. The major advantage of using mice is the wide availability of genetically altered lines or the ease of their generation, allowing the impact of specific genes to be examined far more specifically than achievable with most pharmacological tools. The development of heart failure with cardiac remodeling is a chronic process that is not possible to achieve in the short time span of a terminally anaesthetized animal.

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

All surgical procedures under all Protocols will be conducted under aseptic conditions, with appropriate pain relief, the highest levels of post-operative care and appropriate veterinary consultation. As the major component of mortality or expected side-effects are in the first 24 hours after surgery, animals will be closely monitored at frequent intervals during this period. Animals will be reviewed at the end of the working day on the day of surgery and any considered likely to die overnight will be humanely killed.

Careful attention will be paid to heating, analgesia, body weight, surgical wound-sites, hydration, and signs of pain or distress. Since CHF develops slowly, disease progression needs to be followed for several weeks. The development of heart failure may be associated with loss of weight, listlessness and rapid breathing in the late stages. However, animals will rarely be allowed to progress to such a stage. They will be closely and regularly monitored during the study. Any clinical problems will be dealt with in consultation with the veterinary surgeon. Animals will be humanely killed at a pre-determined endpoint or at the end of the study, whichever happens first.

**What published best practice guidance will you follow to ensure experiments are**

**conducted in the most refined way?**

The AWERB "Guiding principles on good practice for Animal Welfare and Ethical Review Bodies"3rd Edition – September 2015

NC3Rs. Provide online resources and guidelines for 3Rs. (E.g guidelines on humane endpoints and welfare assessments. https://www.nc3rs.org.uk/topic-specific-resources-0

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

Monitoring of NC3 R website, any guidance from AWERB (animal welfare ethical review body) and keeping up-to-date with published literature. Good communication will ensure cascade of information to personal licence holders.

**A Retrospective assessment of Refinement will be due by 14 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?

# 29. African swine fever virus control

**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
    - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

**Key words**

African swine fever virus, Vaccines, Antiviral therapy, Transmission, Immunology

| Animal types | Life stages |
|---|---|
| Pigs | 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.

- 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?**

African swine fever is a lethal haemorrhagic viral disease of pigs for which there is no vaccine or treatment. Through basic and applied research into the disease this project aims to develop tools to protect animals, farmers and global food security from this devastating disease.

**A retrospective assessment of these aims will be due by 02 September 2026**

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

- Did the project achieve it's aims and if not, why not?

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

**Why is it important to undertake this work?**

African swine fever is an invariably fatal disease of domestic pigs and wild boar that can cause the death of infected animals in as little as a week. There is no vaccine or treatment for African swine fever and therefore control is only possible through rapid diagnosis, movement restrictions and slaughter of affected herds. Good farm biosecurity can prevent disease transmission, however a single mistake can lead to disaster and this approach cannot be applied to free-ranging wild boar. Due to the severity of the disease, trade restrictions are applied to countries that are suffering from outbreaks, therefore an African swine fever outbreak within the United Kingdom would inevitably lead to the loss of the export market for pig products, valued at around £500 million in 2018, as well as having a significant impact on the domestic market. African swine fever is now present on four continents and the recent outbreaks in East and Southeast Asia have led to the deaths of millions of animals through disease and culling and has led to a shortage of meat and knock-on effects on food prices. African swine fever has restricted the development of pig farming in sub-Saharan Africa for decades which particularly impacts the rural poor and subsistence farmers. African swine fever is therefore a risk to both the United Kingdom and to global food security.

This project is designed to improve our understanding of the host immune responses that are important for protection against the virus, as well as the mechanisms by which African swine fever virus manipulates host defence pathways. Virus proteins important for inducing protection will be identified, characterised and tested for their ability to induce immune responses in pigs. This data will be used to advance development of vaccines against African swine fever. Antivirals will be tested for their ability to control virus replication in pigs and parameters relating to the transmission of the virus by biting insects will be explored. Taken together this will improve the tools we have available to control African swine fever. This would contribute to the welfare of pigs and wild boar and would limit economic losses for pig farmers and the pork industry. It would also help secure global supplies of pork and pigs.

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

The project will progress work leading to more effective control of African swine fever virus outbreaks. It will lead to increased knowledge of the basic biology of African swine fever virus as well as applied knowledge that may lead to effective vaccines or treatments. The availability of such vaccines or treatments would provide an alternative policy for African swine fever control, avoiding mass slaughter of pigs in the case of an outbreak of the disease. Successful vaccine development is essential to ensure global food security which is threatened by the devastating effects of this fatal pig disease. Pigs provide a stable and cheap supply of high protein food and financial security for many back yard farmers in developing countries and are a main source of protein in many developed countries. A vaccine may even lead to the first steps of African swine fever eradication. Data generated during this project will lead to publications in high impact journals in the field and potentially vaccine or antiviral candidates suitable for commercial development.

This will be achieved by:

Further development of weakened strains of African swine fever virus that could be used as vaccines. This will include comparison of effects of deleting different combinations of viral genes to improve safety and efficacy of these weakened viruses.

Identification of viral proteins involved in protection and defining a minimal number of African swine fever virus proteins that can induce protection against infection with a normally lethal dose of African swine fever virus. Use of compounds that can boost the immune response and different combinations of viral proteins will be tested to improve protection.

Improved understanding of virus host interactions. This will help to define the role of different African swine fever virus genes in manipulating host responses and the host responses that lead to disease. Further benefits will come from a greater understanding of the protective immune response allowing identification of immune correlates of protection, which may allow subsequent development of African swine fever vaccines to be carried out without the need to infect pigs with the virus itself.

The project will generate tools which are applicable for diagnosis of African swine fever, for studies on other porcine diseases, and other basic studies such as pig immunology.

Identification of antivirals that can control African swine fever virus replication in pigs could produce new tools to control disease outbreaks. These could be used either alone to reduce transmission of African swine fever virus and hence limit spread of epidemics, or in combination with vaccines when these become available.

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

African swine fever is a lethal disease and therefore the welfare of pigs would benefit from methods to effectively control the virus. The general public would benefit from new methods to control African swine fever due to increased global food security. Farmers and associated food production and distribution industries would directly benefit, as would governmental organisations responsible for managing these supply chains. Commercial companies would be able to exploit vaccine and antiviral candidates, and suppliers of diagnostic tools could benefit from validation data.

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

Many of the objectives outlined in this project involve collaborative work, either with other research institutes or commercial companies. Results from the studies will be published in high impact open access journals and will be discussed at international conferences. Due to the topical nature of African swine fever it is likely that negative data will be publishable, however in the unlikely event that it is not then draft manuscripts will be uploaded to an online repository such as bioRxiv to ensure the data is made available to all.

**Species and numbers of animals expected to be used**

- Pigs: 480

**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.**

Only domestic pigs, wild boars, feral pigs and four species of African suid - warthogs, bushpigs, red river hogs and giant forest hogs are susceptible to African swine fever virus. The virus only causes disease in pigs and wild boar and the disease in these two animals is practically indistinguishable, therefore pigs are the most appropriate animal to use. There is no small animal model for African swine fever and therefore pigs cannot be replaced. The protective immune response to African swine fever virus cannot yet be studied using tissue culture, isolated organs, non-vertebrate systems or computer modelling.

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

An animal could be immunised with a vaccine and blood samples and nasal swabs taken to see if the vaccine has induced an immune response. The animal could be given one or two more immunisations to act as boosters and blood samples will be taken before and after these immunisations to measure changes in the immune response. In some experiments parts of the immune system may be blocked using specific treatments. These procedures will take place over the course of three to eight weeks.

After this animals are likely to be infected with Africa swine fever virus. Pigs will then be monitored closely for signs of disease and blood samples or swabs taken to study virus replication. Following infection with African swine fever virus, pigs may develop fever, which will consist of high temperatures, lack of interest in food and lethargy, in some cases pigs will suffer increased respiratory rates. Some experiments will involve treatment of African swine fever infected pigs with antivirals.

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

Immunisations, blood collections, swabbing and insect feeding will only cause mild and transient distress that the pigs will quickly recover from and will have no lasting impact.

In a natural situation infection with African swine fever virus would lead to the death of the animal. Through careful monitoring of the animals in our care we will ensure that individual animals suffer at most five days of fever before we intervene and stop the study. However, in a typical study we will intervene after two or three days of fever.

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

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

All the animals will suffer mild severity due to immunisations and/or blood collection.

Animals infected with African swine fever virus may suffer, no adverse effects, mild, moderate or severe clinical signs of disease depending on the study. The proportion of

animals affected will depend on the type of study. For example in a successful vaccination trial most of the animals may suffer no or only mild adverse effects, however if such a trial was not successful then animals may suffer moderate or severe clinical disease.

Naïve, control pigs will develop clinical signs of disease following ASFV infection, which will be limited to moderate severity in the majority of studies.

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

- Killed

**A retrospective assessment of these predicted harms will be due by 02 September 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

The aim of the project is to develop tools to help control African swine fever, a disease of domestic pigs and wild boar. There is no vaccine to help control the disease because classic approaches to vaccine development have not worked for African swine fever. Inactivated virus does not protect pigs and weakened viruses prepared by repeated cycles through tissue culture cause a chronic form of disease. Therefore to develop safe and effective African swine fever vaccines we need to better understand the mechanisms by which the virus causes disease and how the pig's immune response can be stimulated to fight off the virus.
Because African swine fever virus only causes diseases in pigs and virus pathogenesis and protective immunity cannot be studied in the test tube or with computer modelling we need to use animals for this research.

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

We considered cell culture systems, analysis of historic samples and the use of membrane feeding systems for biting flies.

**Why were they not suitable?**

Cell culture and analysis of previous samples will enable us to make reasonable, but not certain, predictions based on previous knowledge and experience about which vaccines or antivirals might be effective. Therefore, we need to test vaccines and antivirals in pigs to prove they are effective. We also need to study the immune response in pigs so that we can improve our predictions about vaccine efficacy.

Cell culture systems cannot replicate the course of disease caused by African swine fever

virus in pigs.

**A retrospective assessment of replacement will be due by 02 September 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 used in each experiment is determined with the help of statisticians to ensure the data generated is scientifically robust, reproducible and uses the fewest animals possible.

The estimate of the total number of animals used in this project is less than the sum of the estimates for the three protocols because animals in Protocol 1 will be moved onto Protocols 2 or 3.

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

Statistical analysis is an essential requirement from our external funding bodies and the project involves professional statisticians who help calculate group sizes required to distinguish between treatment groups based on our previous observations. These observations have established that severity of disease is correlated with maximum levels of virus detected in blood and established the variation of virus titres observed within groups of pigs infected with the same virus by the same route and titre. In some experiments different vaccine formulations will be compared with each other saving the need for a naive control group. Power calculations will be carried out to calculate group sizes required to detect differences with at least 80% power and 95% confidence. In these calculations we will incorporate data from new and previous experiments using either inbred or outbred pigs as appropriate.

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

Extensive use of in vitro and ex vivo assays will ensure that statistically sound results are obtained and will be used to identify correlates of protection that may be used in future to predict pathogenesis of an isolate and/or induction of protective immunity. The calculations will be refined as new data on host responses that correlate with protection and data on levels of virus in protected compared to unprotected or control pigs is collected during experiments.

Animal numbers will also be reduced by using samples that will be collected during vaccination trials which will then be used to complete other objectives within the project.

**A retrospective assessment of reduction will be due by 02 September 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

We will use pigs because they are natural host of African swine fever. Animals infected with African swine fever will be carefully monitored for clinical signs of disease by staff who are trained in animal handling, husbandry and the recognition of signs of pain, distress, disease as well as the ethics of using animals in research. Clinical signs will be recorded daily on score sheets designed to clearly and unambiguously indicate when humane endpoints have been reached. Pigs can be monitored remotely 24/7 if required. Experienced staff will also be responsible for all procedures and so ensure that pain and distress is minimised while procedures are being carried out.

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

There is no less sentient model available for African swine fever virus, therefore we have to use pigs for this research. Small animal models have been tried in the past and were not successful. It would not be possible to follow disease progression in a terminally anaesthetised animal.

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

We will measure immune responses throughout the project. As our knowledge of the protective immune response increases we will be able to make more informed decisions about whether pigs are likely to be protected after infection with African swine fever virus. This will allow us to minimise welfare costs by removing animals earlier from studies.

Animals will be regularly monitored by trained staff and the frequency of this will be increased if the pigs get sick.

All pigs housed within the unit are given positive reinforcement after a procedure to associate a potentially negative experience with a positive one i.e. grapes are given after procedures. The pigs are also given an acclimatisation period to familiarise them with the

staff and routines within the unit. This helps the technicians to also get them used to touch which is needed for taking temperatures without restraint. This is a refinement in animal handling methods to improve animal welfare and the value of animals in research.

Analgesics and non-steroidal anti-inflammatory drugs will be given to animals under supervision of a veterinarian if required.

We will continue to trial the use of pig slings to restrain animals during sampling in order to minimise suffering.

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

We will follow guidelines from the NC3Rs and the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes.

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

By regularly consulting resources available from national and international organisations such as the NC3Rs and from the local Named Information Officer. Discussions on the 3Rs is agenda item of meetings that are scheduled with PILHs after every study.

**A retrospective assessment of refinement will be due by 02 September 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?

# 30. Education of Pharmacology Systems to University Undergraduate Students

**Project duration**

5 years 0 months

**Project purpose**

- Higher education and training

**Key words**

Education, Training, Skills, in vivo, pharmacology

| Animal types | Life stages |
|---|---|
| Rats | adult |
| Guinea pigs | adult |
| 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.

- Education and training licence

## 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 teach University students skills and knowledge of systems pharmacology.

**A retrospective assessment of these aims will be due by 20 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 it's aims and if not, why not?

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

**that accrue after the project has finished.**

**Why is it important to undertake this work?**

It is important to carry out this work because undergraduate pharmacology students can only gain a deep understanding of *in vivo* pharmacology by being involved in real experiments within this subject area. Physical participation in laboratory classes involving animals enables the development of skills in *in vivo* pharmacology. This is important for training the next generation of *in vivo* pharmacologists to support pharmaceutical and university research.

The work will also allow the students to develop their own understanding of how animals are cared for and what the animal's role is in scientific studies. It is almost impossible to develop a deep understanding of how vitally important it is to treat and care for animals in the best possible way (and why this is crucial ethically and scientifically) unless individuals gain some experience of performing experiments involving animals.

**How will course attendees use their knowledge or skills in their future careers?**

Course attendees will be able to use their knowledge or skills to secure positions and develop their careers within *in vivo* pharmacology, pharmacology and related areas. Many students will also be able to use this experience to make a much more informed choice - in terms of whether *in vivo* pharmacology is the right career choice for them. Some students may use the experience to develop a career within the 3Rs sector.

Approximately 97% of graduates are in employment or further study just six months after graduating (DLHE 2016/17). Many of our Pharmacology and Biological Sciences graduates go on to Masters courses in a related subject (a significant proportion of these will go on to PhD positions and other positions in University/Biosciences companies). A significant number of our recent graduates have gone on to directly gain positions within Pharmaceutical companies - a proportion of these were within the field of *in vivo* pharmacology.

Our University also has a strong placement scheme with established links with key pharmaceutical and Contract Research Organisations (CROS). Each year several of our Biosciences students gain placements with these companies.

**What are the principal learning outcomes from the course?**

The learning outcomes from the course closely mirror the objectives of the project:

To develop a strong understanding and knowledge of the systemic factors influencing the nature and magnitude of drug action with regard to pharmacodynamics and pharmacokinetics

To develop a strong understanding and knowledge of drug compartmentalisation

To develop experimental skills in terms of Pharmacokinetic monitoring and analysis

To develop an understanding of the practical application and principles of anaesthetic monitoring

To develop a strong understanding and knowledge of the complex physiological and pharmacological mechanisms required to generate an immunological response

To develop experimental skills in terms of monitoring the body's systemic response to a presented substance (in this case immune response)

To develop a strong understanding and knowledge of the physiological and pharmacological mechanisms involved in generating behavioural responses to agonists and inverse agonists

To develop a strong understanding and knowledge of the systemic physiological and pharmacological mechanisms involved in the development of drug tolerance

To develop experimental skills in terms of behavioural and/or physiological monitoring in rodents

To develop an appreciation of whether *in vivo* studies of biological phenomena in protected animals are essential to obtain knowledge and understanding of physiological and pharmacological principles

To develop an understanding of how to handle experimental animals

A demonstration of best practice in the use of animals for scientific procedures and a culture of care

To develop a strong understanding of the ethical and legal aspects of the use of animals

To develop a strong understanding and knowledge of the principles of experimental design, experimental observation and care in the collection of samples in investigations involving animals

To develop a strong appreciation of the importance of animal care for the integrity of results

To develop skill in the interpretation and critical analysis of the results of research involving animals

To develop an appreciation and understanding of the principles and practice of the 3Rs

To develop an appreciation and understanding of the principles and practice of the ARRIVE guidelines
Most of the learning outcomes are linked to one or more specific protocols and others relate to all of the protocols.

**How are these learning outcomes important to the people on the course?**

To students, developing a deep understanding and knowledge of their subject is very important and these learning outcomes can be readily achieved. Students rightly also identify laboratory skills with employability and these are clearly specified in the learning outcomes.

The learning outcomes linked to caring for animals and developing a culture of care help students to understand that animal research isn't a dispassionate field of work, rather, it

requires compassion, dedication and professionalism. These learning outcomes become increasingly important to students as they proceed through their courses.

We will help our students achieve a standpoint in relation to the use of animals in science and also help our students gain an insight of what skill sets are required for *in vivo* pharmacology. This is crucially important in helping them in deciding what specific jobs and careers they are best pursuing. This will be achieved by associated ethics sessions which provide students with an opportunity to discuss animal research. It is hoped that by supporting our own students in this highly challenging and emotive area we will be helping to equip the next generation to become highly dedicated, compassionate and diligent *in vivo* scientists.

**Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?**

Undergraduate students in terms of skills, training and understanding. These students will have an enhanced opportunity to go on to further positions in relevant pharmaceutical companies and universities. Relevant opportunities will be in the areas of pharmacology, related science and the 3Rs.

Employers who will be able to find qualified graduates with in depth understanding and skills in *in vivo* pharmacology. In addition, employers will benefit from recruiting graduates who will have been able to develop an informed standpoint on the subject area of 'Animals in Science'.

Discussion with key pharmaceutical and contract research companies tells us that these companies highly value the *in vivo* training in pharmacology that we can provide our graduates.

The wider economy and life sciences sector will benefit from the outputs produced by trained *in vivo*
pharmacologists.

The healthcare system will benefit in the long term from the knowledge and treatments produced by these trained individuals.

**How will you look to maximise the outputs of this work?**
We will seek to optimise and improve the educational and training aspects of this work via feedback and conversation with students. Students provide the best guide in terms of how well we are addressing our objectives. In our previous licence we have had very positive feedback from a high percentage of students (~80% or higher). The students find the laboratory classes interesting and stimulating, they improve their understanding of the subject area and the students appreciate how the classes are directly relevant in terms of skills training (particularly in relation to *in vivo* pharmacology jobs).

Another way we will maximise the outputs is by facilitating related ethical teaching associated with the *in vivo* pharmacology teaching. This aspect specifically relates to future careers in drug development, medical research, biomedical ethics, clinical trials and the development of new 3Rs related technologies.

*Students participate in these classes by monitoring animal physiology and behaviour and in some cases handling animals. Supervision of students depends upon the specific lab*

*class. For behavioural analysis classes we have a staff:student ratio of approximately 1:8 (protocol 3). When classes involve physiological or anaesthetic monitoring the staff:student ratio is at approximately 1:8 (protocol 2 and 4) and 1.7 (protocol 1) respectively. Class sizes range from 20-35 students at one time depending on the protocol.*

*We will also use interactions with employers and previous students to maximise impacts and outputs from the licence. This will be achieved by facilitating discussion with employers and previous students via*

*personal communication and social media contact between Pharmacology Team staffwith graduates and employers*

*meetings such as the 'Employers Talks' events organised by the Employability Team at our university.*

*To maximise our outputs we will follow and align our teaching content with the curriculum for the use of research animals published by the British Pharmacological Society (November 2020).*

We will also seek to share best *in our local* AWERB and beyond within the University e.g. ethical teaching in other subject disciplines within the University.

**Species and numbers of animals expected to be used**

- Mice: 500
- Rats: 50
- Guinea pigs: 35

## Predicted harms

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

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

Rats are used because they are a mammalian species with a similar circulatory and metabolic systems to humans. They are large enough to allow relatively straight forward analysis of compartmentalisation and pharmacokinetics.

Guinea pigs are chosen because they have a similar immune function to our own and are a good model of type 1 hypersensitivity. They are also easy to work with and handle.

Mice are used because they have similar behavioural response to benzodiazepines and it is relatively easy to monitor behaviour in these animals.

Mice are used because they have similar metabolic systems and responses to barbiturates. In addition, they also develop drug tolerance similar to that seen in humans.

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

Animals will receive intraperitoneal injections of anaesthetic, a tail cannulation and an injection of dye intravenously. After the first injection of anaesthetic animals will be under

anaesthesia for the whole protocol. The duration of experiments will typically be under 2 hours.

Animals will receive an intraperitoneal and/or injection of albumin and then approximately 2 weeks later, in some instances, an injection of antihistamine followed by skin test via intradermal application of albumin and monitoring of skin reaction by students. The antihistamine and skin test will be typically last under an hour.

Animals will receive an intraperitoneal injection of inverse agonist drugs. They will then be placed on a hole board and their behaviour monitored by students. The injection and hole board test will take less than 1 hour.

Animals will receive an intraperitoneal injection of a sedative drug on day 1 and day 8 and an intraperitoneal injection of a barbiturate like compound (TCPOBOP) on day 7. Sedation will occur after the injections of pentobarbitone and students will monitor the levels of sedation. Sedation will typically last for about half an hour.

All regulated procedures will be carried out by experienced PILhs (animal care staff) rather than the students.

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

Possible risk of anaesthetic levels being too light or deep, this risk will be managed via monitoring of physiological parameters and reflexes such as breathing rate, eye blink reflex, pedal reflex and tail reflex and necessary anaesthetic 'top-ups' will be applied in line with veterinary advice. Possible risk of hypo/hyperthermia, this will be managed by monitoring body temperature and they will be placed on a heat pad.

Expected skin reaction to intradermal application of albumin in some animals. There is risk that the skin reaction could develop above the specified threshold. If the skin reaction reaches the threshold animals will be euthanised immediately via an S1 method. There is a small risk (<1%) of seeing of anaphylactic reaction in response to the application of egg albumin. If anaphylaxis is observed the animal will be immediately killed via a schedule 1 method. To mitigate this risk animals will be sourced from Barrier Units and will be fed albumin free diets.

For behavioural analysis there are no other expected impacts as the hole board is at a relatively low height and thus produces a baseline level of exploratory behaviour in mice; the drugs will be employed at low concentrations which produce subtle changes in behaviour.

There is a small risk of hypothermia during or after sedation. To prevent hypothermia animals body temperature will be monitored and they will be placed on a heat pad.

**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)?**

Rats - non recovery - 100%. Guinea Pigs - mild - 100% Mice - mild - 100%

**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 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

It is necessary to use animals to teach students knowledge and skills in *in vivo* pharmacology because students need to see and gain 'hands on' experience in these experiments to develop detailed knowledge, deep understanding and skills in this field.

**Why can’t your aim be met by observing or by participating in ongoing research or clinical procedures?**

Observation or participating in ongoing research or clinical studies would not provide this concise and quite comprehensive overview as the projects would only cover a focused project. The same projects could not be available to all, to produce a consistent experience to all and would not be feasible in terms of providing the necessary close and hands-on training (without a personal licence) required to develop skills, a culture of care and wider perspective on ethical aspects of animal use in science. For this an external course provider delivers the following modular training to the students; L, E1, PILA (theory and skills), PILB, K (theory and skills) and rodent handling.

The proposed scope of our licence gives students a broad overview of key areas of in vivo pharmacology in terms of fields and skills. The fields covered include pharmacokinetics, systemic responses and the immune response, behavioural pharmacology and drug tolerance and metabolism and addiction. This broad and comprehensive overview could not be achieved by participating in ongoing research or clinical procedures.

**A retrospective assessment of replacement will be due by 20 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 yourexperience?

## Reduction

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

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

I have estimated the number of animals I will use based on previous and similar numbers of animals required to produce significant results and optimum student group and class sizes. In addition, I have used the previous numbers in each student year. Generally, student group size range between 3-4 students.

**What in silico or ex vivo techniques will you use during training?**

During training we will use the following *in silico* or *ex vivo* techniques:

*In silico* training:

- organ bath simulations
- simulations to model drug administration and elimination

*Ex vivo* techniques:

- videos to prepare students in terms of what they will see and how to observe behaviours and physiological parameters.
- dissection classes, anatomical classes (sometimes using anatomical models) and histological classes
- organ bath classes

We employ animal handling prior to the start of these protocols.

**Will these techniques reduce animal numbers? If so, how?**

*In silico* training allows aspects of pharmacology and physiology to be taught without animals, which reduces the number of animals used in teaching laboratories - both *in vivo* and *ex vivo*. It also allows virtual training prior to laboratories involving animal tissue (*ex vivo*) or *in vivo*.

*Ex vivo* approaches can be used to train students prior to *in vivo* laboratories. Being better trained for laboratories allows students to understand the labs more clearly, produce better results in the laboratory and take a more professional approach to the laboratory. This results in better results obtained and better and more successful completion of learning outcomes and less animals used.

**What other measures will you use to minimise the number of animals you plan to use in your project?**

We will use optimised methods to ensure we obtain the maximum amount of data from the number of animals used.

We will pool data from within and across teaching classes so that individual student groups

partake in a portion of the entire experiment. This ensures that each student can experience the laboratory and training, and also ensures that the minimum number of animals are used.

We will manage the number of animals used per given number of students. For example, we will use the minimum number of animals required according to the size of the teaching class - this will be managed at group, class and year level.

**A retrospective assessment of reduction will be due by 20 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.**

Rats will receive a cannula into their tail vein. During this process they will be anaesthetised therefore they will be insentient throughout. Tail vein cannulation is a much less invasive approach than other administration methods such carotid/femoral artery cannulation.

We will induce type 1 hypersensitivity and test for a mild skin reaction in guinea pigs. We will minimise any distress by using standard, simple injection procedures and carefully monitor any skin response to minimise any discomfort or harm.

For behavioural analysis of mice we will minimise distress by giving a single injection and minimise arousal or stress by using a hole board at low height when recording locomotory responses.

To teach about drug tolerance we will use simple injections to give the drugs to mice. This will minimise discomfort. When sedated we will provide a heated pad to ensure that the animals don't become too cold.

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

In all of the protocols we need to use mammals to accurately model the drug action which is seen in humans i.e. in terms of pharmacokinetics, immune responses and behaviour and drug metabolism.

Rats are a larger rodent (than mice for comparison) which makes the study of pharmacokinetics and compartmentalisation much easier to teach. The rats will be

anaesthetised throughout therefore they will not be sentient.

Guinea pigs are excellent models of hypersensitivity and regarded as better models than other rodents such as rats and mice. Guinea pigs are one of the least sentient mammalian models.

In our behavioural analysis, we will use mice which display very similar drug responses to humans and are one of the least sentient mammalian models available.

In our drug tolerance experiments we will use mice which show similar mechanisms of drug tolerance and addiction as humans and mice are one of the least sentient mammalian models available.

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

In the Pharmacokinetic experiments it is possible we will see some haemolysis in the terminal blood samples taken. We will minimise this by continually refining our blood sampling technique. We are planning to refine this protocol by employing new fluorescent markers – this could replace the existing marker/dye.

For the Hypersensitivity experiments, typically type 1 hypersensitivity is induced by giving 2 injections. Over the course of this licence we hope to reduce this to one if the results produced are of adequate quality. Pilot work will be used to optimise and refine the skin allergy test.

In the inverse agonist (behaviour) experiments it is important to get the dose of the drugs correct so that the results are the most meaningful and so that the best possible use of the animals is achieved in relation to teaching objectives. In addition, it is also necessary to get the correct dosage so that the experience of the animal is the best possible one and the associated impact is minimised. In this laboratory we will optimise the dose for diazepam to maximise anxiolytic effect whilst at the same time reducing any signs of sedation. We will also optimise the dose of the inverse agonist used to generate the best behavioural and welfare effects on the animals.

In the drug tolerance experiments optimisation of pentobarbitone dose level is of crucial importance. This is because we seek to induce loss of righting reflex, but we want this sedation to be mild. In addition, we also seek to demonstrate dose dependence of pentobarbitone action therefore there is a relatively small dose window we wish to work within. We will monitor doses of pentobarbitone carefully to check that they are optimal.

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

I will follow published best practice guidance provided by the NC3Rs (UK), the Norway's 3R centre and National Consensus Platform for the 3Rs (NORECOPA) and the Laboratory Animal Science Association (LASA).

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

I will stay informed about advances in the 3Rs via NC3Rs bulletins, local University

AWERB, literature search of published papers, via the NC3Rs website and local discussion with academics and AWERB related staff.

Continuously we will review any advances in relation to applying them to the programme of work.

**A retrospective assessment of refinement will be due by 20 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?

# 31. Production of Antiserum

**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**

Pathogen, Vaccine, Antibody, Antisera, Diagnostics

| Animal types | Life stages |
|---|---|
| Mice | adult |
| Ferrets | 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.

- Required at inspector’s discretion

## Objectives and benefits

**Description of the projects objectives, for example the scientific unknowns or clinical**

**or scientific needs it's addressing.**

**What's the aim of this project?**

The objective of this project is to produce reagents (substances used for biological or chemical analysis) and data for the diagnosis, control, or prevention of communicable (and other) diseases. The reagents are produced using animals in experimental procedures to produce antisera which is then used in non-animal laboratory tests for the control, prevention, surveillance, and diagnosis of disease.

**A retrospective assessment of these aims will be due by 14 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?**

Antisera is used in the diagnosis, control, surveillance, and prevention of communicable diseases such as Influenza (Flu) and other viral or bacterial diseases.

This antiserum is not commercially available on the market and is used as an integral component to surveil and diagnose current, new, and emerging infectious diseases.

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

The outputs will be data and knowledge used for the diagnosis, control, or prevention of communicable (and other) diseases.

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

The benefits will accrue to the population of the UK (directly) and globally through our participation in infectious disease networks. The work generated from this project will directly contribute to vaccine development which leads to positive public health outcomes.

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

Global network participation, presentations and publications are routine mechanisms.

These publications inform both national and international agencies insiders and the public of the progress and benefits of our work such as surveillance of influenza and other respiratory viruses in the UK which will be shared via digital platforms.

**Species and numbers of animals expected to be used**

- Mice: 200

- Ferrets: 200

## Predicted harms

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

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

The animals that will be used in this protocol are used because they are the most appropriate species for the test. For example, ferrets are particularly susceptible to influenza (flu) virus and therefore are a vital part of learning about the disease.
It is more advantageous to use young adults rather than infant or older animals as young adults are at the peak of their physiology and less likely to suffer from age-related illnesses or organ immaturity which can be problematic and is a possibility if immature or aged animals are used.

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

Animals will be given an inoculum that will elicit an immune response i.e., antibodies will be generated to protect against that disease.

The antibodies will be collected and used for diagnosis, surveillance, control, and prevention against communicable diseases.

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

The impact on the animals during the project is mild and any pain, suffering, and distress will be transient.

We do not expect any animals to exceed the prospective severity of the protocols.

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

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

The severities of this project are mild, and any pain, suffering, and distress experienced will be transient
e.g., slight malaise.

We expect all animals to have a mild experience under these protocols, this is because the procedures that are being performed are inherently mild in nature.

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

- Killed

**A retrospective assessment of these predicted harms will be due by 14 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?**

We currently use animal-derived polyclonal antibodies for use in a laboratory setting for the diagnosis and surveillance of communicable disease.

This work would not be possible without the use of animals that are needed to produce the antibodies.

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

We currently use next-generation sequencing (NGS) and whole-genome sequencing (WGS) for genetic characterisation (identifying the genetic make-up) of influenza (Flu) virus, which is an important part of the process. However, this does not aid us with the antigenicity (capability to bind to an antibody) testing or antibody titration i.e., antibody concentration, these tests depend on antisera.

Non-animal derived monoclonal antibodies (MAbs) are commercially available and will be used if needed.

**Why were they not suitable?**

We cannot solely rely on NGS and WGS because it does not aid with antigenicity or virulence information i.e., the severity or harmfulness of a disease.

To ascertain the harmfulness of a virus and the capability of a virus to bind to an antibody, antisera is required.

Currently, there are no suitable or reliable technologies that can recreate the broad polyclonal antibody specificities elicited by immunisation of a mammalian host.

**A retrospective assessment of replacement will be due by 14 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?**

These numbers are based upon the experience of 40 years of using the techniques to determine animals to be used in a 'normal' non-pandemic or routine use scenario plus additional that would be used to consider a spike in infections across the UK.
These numbers provide a surge capacity that enables us to provide a critical service in the prevention and control of transmissible diseases.

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

We use the minimum numbers of animals to meet health protection outcomes. Our use is driven by infectious disease surveillance, prevention, and control, rather than the statistics of experimental design. At the time of writing this application, we have used less than half of the estimated number of animals under the current licence.

In the event of an outbreak, the number of animals used would increase dramatically over routine use as a response to the public health emergency.

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

Animals are procured at point of need to keep the numbers to a minimum and ferrets that are on the normal blood project licence can be re-used on this project once they have passed a health assessment carried out by the NVS.

**A retrospective assessment of reduction will be due by 14 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.**

Mice and ferrets will be used during this project.

Mice generate an excellent immune response to vaccinations given. We use minimal volumes in the vaccinations which are tested for sterility and purity, and we only require a

small volume of antisera from mice, this is a refinement and progression from using rabbits for antisera production in the past.

Reduced volumes of blood are taken for the pre/test bleed because laboratory analysis techniques are now more efficient and require reduced volumes to produce a valid result than previously, therefore reducing the overall impact on the animal.

It is well documented that ferrets are extremely susceptible to influenza (Flu) which makes them the ideal model for influenza work.

The pain, suffering and distress the animals' experience on this project is minimal and the prospective severity is mild.

Mice are given a vaccination via a parenteral route i.e., injection using a hypodermic needle and a ' test 'blood sample is taken via the most advantageous and painless route for the species.

Large blood samples are performed under general anaesthesia and intranasal vaccinations are also given under general anaesthesia.

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

We must consider the maturity of the immune system to be able to produce viable antibodies. Immature animals are not able to produce antibodies of the required specificity at the volumes needed.

Animals are terminally anaesthetised to collect large volumes of blood from the heart, this is the most humane method to collect large volumes of blood in a single event.

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

- The procedures are already well refined from experience over many years of their use. We always aim to minimise pain, suffering, distress, or lasting harm.

- We use the most appropriate routes of administration for vaccinations and use anaesthesia where the use of anaesthesia will cause less harm than the procedure itself.

- Post-procedure observations are carried out and the animals are monitored throughout the project
- e.g., via strict weight monitoring and health assessments.

- Ferrets also benefit from human interaction which they receive daily.

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

We follow best practice regarding experimental techniques such as vaccination and blood-letting techniques from literature.
The above literature gives detailed guidance on animal management, welfare, and the correct/most refined methods of experimental techniques.

I must emphasise that the experimental techniques used under this project consist of vaccinations and blood-letting which when conducted competently are always of a mild severity that causes little to no pain, suffering, distress, or lasting harm.

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

- We are signed up and receive newsletters informing us about advances in animal science. I am looking to advance the refinement through environmental enrichment programmes and the animals are floor housed where appropriate.

- I am a member of the Institute of Animal Technology and receive regular correspondence.

- I stay informed via discussion with the NVS and through networking with colleagues from other organisations.

**A retrospective assessment of refinement will be due by 14 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?

# 32. Stem cell function in cancer

**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**

cancer, stem 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?**

We aim to understand and explore exactly how cells in the body become cancerous. We will also investigate the ways in which cancer cells are able to survive treatment with drugs designed to kill them or block their growth. We hope our research may ultimately lead to the creation of more effective treatments that tackle the unique features of cancer and which are harder for cancer cells to resist.

**A retrospective assessment of these aims will be due by 19 September 2026**

The PPL holder will be required to disclose:

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

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

**Why is it important to undertake this work?**

Cancer remains one of the leading causes of death worldwide. Although there has been considerable progress in the treatment and management of several cancer types in the past few decades, others have remained largely unaffected by the latest advances in treatment such as new immunotherapies, whereby the body's own immune system is recruited to kill the cancer cells.

A key example of a cancer that remains very difficult to treat is pancreatic ductal adenocarcinoma, which is the fourth most common cause of cancer-related deaths in the Western world and has a five- year survival rate of only 30%. We aim to investigate this cancer by looking at how it arises and what makes it particularly resistant to treatment. By doing this we hope therefore to develop more effective therapies for this and other hard to treat cancers such as squamous cell lung carcinoma and intestinal cancer.

Cancers are thought to arise from a type of cell known as a stem cell. Stem cells have the unique ability to develop into the specialised cell types required for a particular organ to function. In healthy tissues, they are used to replace cells that have been damaged or lost due to disease. However, in cancer they gain mutations that allow them to grow and divide uncontrollably. Because these stem cells are able to develop into many different cell types, cancers are very diverse in nature and this diversity is believed to be one of the main causes of tumour aggressiveness and resistance to therapy. Our project will explore how stem cells that develop into pancreatic cancers contribute to the disease's genetic diversity and its ability to become resistantto drug treatment.

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

We expect to make important advances in our understanding of how cancer originates and develops. Our approach has achieved results which have led to numerous publications in top-tier peer-reviewed journals, and we are confident that we will continue along these lines, and learn more about the cellular and molecular mechanisms at work in tumour biology. In addition, we believe we will be able to open up new approaches to cancer treatment. Under the previous licence, we identified an enzyme that degrades proteins as a possible therapeutic target in intestinal cancer. These findings are now being used by a pharmaceutical company that has started a programme to discover and develop drugs that block this enzyme for cancer therapy. In addition, we have identified a protein on the cell surface which is important for pancreatic cancer initiating cells and we are working now with industrial partners to create new therapies using this target.

Expected outputs derived from this PPL:

A large part of our work is based on the various roles of stem cells in disease. Stem cells have the unique ability to develop into the specialised cell types required for a particular organ to function. We expect to provide novel information about how stem cells are able to divide indefinitely to produce more cells of the same cell type and also the cellular signals that can cause a stem cell to give rise to intestinal, lung and pancreatic cancer.

Understanding more about stem cells will advance our knowledge of the cellular processes that control tissue function and recovery from injury, and how these can malfunction in cancer. We aim to understand the molecular basis of the systems controlling tissue regeneration and the development of cancer, and to understand whether we can turn the activity of these systems up and down. We hope this work could open up new treatment strategies and be useful for the discovery of new drugs for treating cancer.

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

The overall aim is to gain an understanding of the key pathways involved in the development of cancer that will allow us to identify novel drug targets for their future use for patients in the clinic.

Who will benefit:

Basic science – The novel information acquired from this proposal will have a direct benefit for both national and international academic institutions that are carrying out research into cancer.

Clinical translation: The results from preclinical models will be beneficial to clinicians as one of the aims of this proposal is to address cancer's resistance to treatment. Understanding drug resistance will help clinicians in making decisions about how to treat patients more effectively and in the design of new treatment strategies.

Patient benefits: We aim to create novel therapies as part of this proposal for patients with advanced cancer for whom all other therapies have failed. The overall survival in pancreatic cancer has not improved over the last 30 years. This is in sharp contrast to lung, colon and breast cancers where the overall survival and life quality has significantly improved over the last few decades. We hope to be able to benefit patients with pancreatic cancer by identifying novel drug targets and thereby new treatments that could be used instead of or in combination with standard chemotherapy in pancreatic cancer treatment.

How information will be disseminated: The information will be distributed through a wide range of channels including presentations and posters at relevant conferences, organisation of workshops, publications in relevant journals. In addition we will also announce breakthroughs and updates through social media channels such as Twitter and the institutional website.

Expected short- and long-term benefits

Short term:1. The discovery of critical molecules and pathways in the development of cancer will be used to guide the creation of new therapeutic agents. 2. The therapeutic potential of these agents will be established and assessed in different animal models. We will combine the novel drugs with the standard treatments and compare the outcomes. We will also identify the side effects of new therapeutic agents and monitor for potential safety concerns.

Long term:1. The information we get regarding the mechanism of actions and safety of novel drugs we identify will help expedite their approval for use in the Clinic by governing bodies such as the Food and Drug Administration (regulatory body that approves new medicines for use in America). This will help new drugs to be quickly translated into a clinical setting.

2. This project will provide data about the novel therapeutic treatments that will potentially be used in the clinic, and offer guidance in selecting patients for clinical trials depending on the cancer type. In addition, the results from this work will also help in monitoring therapeutic resistance (when drugs become ineffective against a disease), potentially providing more effective cancer therapies.

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

This project is a continuation of previous work which has provided a series of new therapeutic targets that are currently a focus of drug development strategies. It has also led to the production of highly cited and impactful publications. Our previous work also facilitated numerous collaborations with many UK and European institutions as well as clinical translation. Due to the diverse nature of this project, this proposal will enhance the specialised collaborations among different disciplines. These collaborations will include the use of mathematics where data sets derived from our drug screening would be used to make mathematical models of tumour growth and behaviour that may predict therapeutic response. The dissemination of new knowledge will be through appropriate channels for different academic and clinical backgrounds. This will be done via presentation at national and international meetings such as at the American Association for Cancer Research meeting which will stimulate further collaboration.

The transgenic animals developed in the course of these projects will undoubtedly be valuable to other scientists interested in the development of anti-cancer therapies and we will freely share these lines.

**Species and numbers of animals expected to be used**

- Mice: 90000

## Predicted harms

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

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

The mouse is the most appropriate model species for this investigation as they are the lowest animals in the evolutionary tree in which suitable models of cancer treatment can be carried out. The project requires genetically modified mouse strains that are immunocompromised to assess the distribution of human cell lines to prevent rejection by the mouse immune system or to be able to spontaneously generate genetically diverse tumours. It is important to test new therapeutic agents within human cell lines because in vivo therapeutic response is not consistent across cell types (e.g. adenocarcinoma versus squamous cell carcinoma) or across animals (e.g. cells derived from mice versus humans). It is also important to test targeted therapies in a genetically diverse tumour as the efficacy of targeted drugs is directly related to the range of protein molecules produced by the cancer cells. All experiments will be conducted in adult mice.

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

1) In general mice will either (a) be bred with mutations that induce tumour formation or

(b) will receive an injection of tumour cells to induce tumour formation, which may be via a surgical procedure or (c) by administering compounds that induce tumour formation or tissue damage. In a limited number of cases, some mice will undergo surgical procedures. All surgical procedures are short procedures (up to 20 minutes). The use of surgical procedures has also been refined by the use of ultrasound-guided techniques where possible. This results in less tissue damage during surgery to aid animal recovery.

2) Animals are scanned using non-invasive imaging (1 to 2 times weekly up to 1 hour) to monitor tumour development. Once tumours have reached treatment size (typically within 2-3 weeks) the animals will be randomised into groups for treatment.
3) Tumour-bearing animals will be given either single or multiple doses of therapy (cell or drug) dependent on the route of injection e.g. intravenous may be given multiple times (dependent on the dosing table) whereas injections via a surgical route will be given only once.
4) Animals will continue to be monitored non-invasively via imaging (see above) to follow treatment outcomes such as changes in tumour size and tumour cell death. The typical duration of experiments will be 1 to 2 months depending on the growth rate of the tumour.

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

Animals will experience tumour development and tumour treatment and may experience weight loss. Body weight is monitored twice a week and any mice showing a 10% weight loss will be monitored daily. If they experience 15% loss (in total) over 48 hours, they will be humanely killed by a schedule 1 method. The body condition and behaviour of animals will also be assessed.

Surgical procedures and cell injections can cause internal bleeding or vascular occlusion. Animals are assessed rigorously during procedures and directly after upon recovery, and any animal showing evidence of bleeding or vascular occlusion will be humanely culled immediately.

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

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

The expected severities are moderate and mild. We expect that around 85% of the mice will be in the mild category as these are the animals that will be used for breeding and maintenance.

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

- Killed

**A retrospective assessment of these predicted harms will be due by 19 September 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?**

There are several reasons why our research cannot be done without using animals:

1) At present there is no system to mimic tissue repair using cells grown in the lab.
2) Stem cells inside the body are not only dependent on their specific environment (niche) but also do not usually replicate. In cell culture conditions, many stem cells are constantly dividing and thus do not represent stem cells in their physiological condition.
3) Many kinds of cells, including stem cells, spontaneously accumulate mutations when cultured in the lab which increases the risk that the effects observed are due to random mutations rather than the genes we are studying.
4) To validate candidate genes as potential anti- cancer drug targets, it is essential to analyse their function in tumour development inside the body.

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

Prior to embarking on animal experiments we performed preliminary screens (for example using inhibitors of genes of interest) in a 3D cell culture platform. This uses cells derived from patient's tumours grown in a 3D matrix that mimics as closely as possible the physiological situation. Only those inhibitors that worked in this non-animal model are taken forward for mouse studies. However these preliminary studies alone are not sufficient to fully evaluate a drugs effectiveness or safety in the body
**Why were they not suitable?**

The complexities of human physiology cannot be easily recapitulated in non-mammals. Lungs, intestinal and pancreatic cancer are complex diseases that are caused by an intricate interaction of many different cell types. For example, pancreatic cancer cells are known to have a strong interaction with surrounding normal cells, the so-called stroma, and this interaction is important for tumour formation.

**A retrospective assessment of replacement will be due by 19 September 2026**

The PPL holder will be required to disclose:

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

## Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise**

**numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

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

We have based this number of animals largely on previous experiments and the literature. We have extensive prior experience using these models and therefore have a good idea of how many we require to achieve our aims. Based on our previous data we will use power calculations to determine the minimum number of animals required to give a statistically meaningful result.

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

All animal studies are designed with assistance from the NC3Rs Experimental Design Assistant and using PREPARE guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence; doi: 10.1177/0023677217724823). We will consult with a statistician with regards to the experimental design to minimise the number of animals used whilst ensuring meaningful data can be collected. We always strive to improve novel technologies to refine our experiments, in order to create in vitro conditions, which closely match those found in vivo, therefore reducing the total number of animals necessary for our studies. For example, when possible, experiments will be performed using cell lines in vitro. We have developed organoid systems for the pancreas and the intestine to study cell function. Organoids are a miniaturised and simplified version of an organ that can be grown in the lab and used to mimic tissue function. We will manipulate the expression of genes we are interested in to study their function, reducing the number of genetically engineered mouse models required. A small number of experimental mice, typically not more than 3-5 mice per group, will undergo non-invasive imaging to analyse tumour size and location without the need to sacrifice the animal. Response to treatment will also be monitored by non-invasive imaging method, for example ultrasound, to reduce the animal numbers because each animal can be used as its own control and allowed to make paired comparisons. Monitoring the same animal at different time points of the treatment will provide us with more information while using fewer animals as different groups of animals are required for each time point in conventional designs. In addition, sequential experimental designs will increase statistical power compared with conventional designs.

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

When we are breeding mice, we will maintain those strains that are only used sporadically at lower levels, and freeze embryos whenever practicable. Strains will be maintained in collaboration with other licencees wherever possible to minimise unnecessary breeding. We will typically keep mouse colonies to a minimum size (2-4 breeding pairs depending on breeding performance, genetic background and viability). Whenever possible, these strains will be kept as homozygotes to reduce wastage. We use advanced molecular technology to generate mouse colonies with gene modifications in cell populations of interest rather than the whole mouse. Such technologies will also enable us to reduce the number of mice used in their generation and in experiments. In addition, we transplant genetically modified tumour organoids into relevant organs to develop more physiologically relevant animal models and to cut down the need for breeding complex multiple alleles. For tumour studies, we aim to use the minimum number of mice per group that will be

informative. The animal numbers required to obtain significant results are dependent on the particular tumour model. The most important parameter is the reproducibility and thus predictability of tumour development of a particular model. We use cohorts of 10-15 mice for tumour models where tumour development occurs in 100% of the animals.

These models mimic human inherited cancers, i.e. where certain families always develop a certain tumour type. We typically study 20-25 mice per genotype for tumour models where tumour development is random and not all animals develop tumours. These models mimic sporadic cancers, which account for the vast majority of cancers in the human population. To maximise the information from a single animal, we aim to collect samples from multiple sites in the body after a mouse has been killed and provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. In addition, tissue from multiple organs will be analysed by using a three-dimensional imaging technology which we recently developed in the lab. By using this technique, more comprehensive information can be obtained from a single animal, reducing the number of animals required for studies. We will analyse and evaluate results obtained from animal experiments using statistical software.

**A retrospective assessment of reduction will be due by 19 September 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

The mouse is the most suitable model species to study genetic mutations related to cancer as its genetic landscape is very similar to humans, and yet it is relatively low in the evolutionary tree. In addition, using mice is an obvious choice because of the range of readily available mutant mouse models and established techniques for further manipulation of gene expression. The animal models we have chosen are well characterised and well documented to produce reliable results while only having moderate effects on the mice. We use well-established methodologies that we know have little or no adverse effects by themselves to activate or inactivate specific genes. We will then monitor mice for signs of tumour development, typically including weight loss, inactivity or sometimes other specific characteristics of a particular tumour type.

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

For studies of cancer, we need to use adult mice as this is more physiologically relevant. In addition, we will be studying how cancer grows and responds to treatment over a period of time that could be as long as six months, so the use of young or terminally anaesthetised

mice would be impractical.

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

To generate transgenic mice, where possible we will use mutations that are inducible (the mutation only becomes effective if activated by a drug given to the mouse, such as tamoxifen) and conditional (the mutation is only expression is specific cells or tissues). That means that mice should not display an observable effect of the mutation except in particular circumstances such as when the mutation is induced. We predict from previous cell culture experiments or publications that the expression or deletion of candidate genes is unlikely to result in severe effects on the mice. However, to avoid unexpected pain and suffering, animals will initially be bred and analysed as heterozygous animals (only expressing one copy of the gene in question). We will only use well-established reagents and protocols to induce expression or deletion of the candidate gene. Where the gene mutation or deletion leads to cancer, we will apply growth inhibitors (for example, potential anti-cancer drugs). When the toxicity of the agent is unknown we will use the lowest dose on a minimal number of animals. By choosing well-established protocols to induce tumours we minimise unknown effects on the mice and consequent pain, distress and suffering. Our aim is to identify drug targets for potential new anti-cancer drugs, and we will only subject animals to cancer mouse models when we have sufficient in vitro and in vivo evidence that the candidate or treatment might protect them from tumour development. Animals undergoing surgical procedures will receive anaesthesia and post operative analgesia. After surgery, the animals will be intensely monitored until they have recovered from the anaesthesia. Then, animals will be monitored every 20 minutes for the first hour, then every 1-2 hours within working hours and first thing the next morning. If no clinical signs are shown and the wound shows no swelling or bleeding, the animals will be monitored 2 to 3 times a week. We are continuously developing technologies to refine our experiments and to minimise suffering of our research animals. Non-surgical ultrasound-guided injection will be performed in some groups of animals to induce tumours or deliver drugs. This reduces any risk to infection, greatly increases the rate of recovery and limits pain.

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

We will follow the guidance given in the NC3Rs ‘Resource Hub’ (https://nc3rs.org.uk/resource-hubs) for example on blood sampling (https://www.nc3rs.org.uk/blood-sampling-mouse) and effective use of genetically altered mice (https://www.nc3rs.org.uk/GAmice). We will also refer to the National Cancer Research Institute guidelines on using animals in cancer research published by Workman et al 2010 (British Journal of Cancer 102, 1555 – 1577).

**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 the latest developments on refining animal research methods via the NC3Rs website (https://www.nc3rs.org.uk). Animal house staff will ensure that any advances are fully implemented throughout the facility.

**A retrospective assessment of refinement will be due by 19 September 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?

# 33. Adoptive cell transfer for cancer immunotherapy

**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**

Cancer, Immunotherapy, Chimeric antigen receptors, Adoptively transferred T cells

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

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is 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?**

T cells are blood cells that help fight infection or even cancer. This project focuses on the development of therapeutic T cells for the effective and safe treatment of cancer.

**A retrospective assessment of these aims will be due by 18 July 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?**

Therapeutic T cells and more specifically chimeric antigen receptors or CAR T cells, are one of the most important medical breakthroughs of a generation. This treatment is based on taking blood cells from a patient to obtain T cells, modifying these cells in the laboratory and then giving these cells back to a patient by drip where they are able to attack and kill cancer cells. However, to date, CAR T cells are only effective in a few cancers, are very expensive to make and patients can experience severe side effects. The ultimate goal of this work is to develop CAR T cells that are able to effectively treat cancer patients. This is not possible without assessment of CAR T cells in animal models as assessment in test tubes alone, or computer simulations would not allow us to understand their behaviour in complex biological systems- essential to ascertaining the potential of CAR T cells for patients.

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

Our research aims to develop the next generation of CAR T-cell therapies for cancer and other conditions. Our work will increase the number of patients who will benefit from CAR T-cells, and to also make CARs safer. Thus, our research ultimately aims to save lives and improve the health of the population.

Other benefits of our approach may include economic value. By inducing long-lived remissions in patients we may reduce the burden on healthcare systems and a ‘one-off’ treatment such as CAR Tcells may ultimately prove less expensive than current modalities.

As part of our research, we will disseminate our work via publications in the scientific literature, presentations at conference meetings and public engagement activities. Thus, we aim to benefit the field, including other academic and industrial researchers, and ultimately patients, by increasing the knowledge in this area. Further, we aim to develop new technologies which may be used not only by us but by other groups both in the CAR and broader gene-therapy fields.

Further, the individuals conducting the planned research will develop new skills, knowledge and understanding which they will use to further benefit both the scientific community and society as a whole.

In summary long term benefits include developing this therapeutic strategy cancer to address an area on unmet clinical need with the potential to benefit individual patients and provide economic benefit to healthcare providers. In the short term, this will work will contribute to the scientific community and increase knowledge in the field and provide vital training for the next generation of cancer researchers.

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

The ultimate aim is that CAR T cells will be developed that will have a positive impact on patient care. In the last 5 years at least 4 clinical trials have opened directly from CAR T

cells developed at this establishment thus it is expected that CAR T cells will progress from testing in mice to use in the clinic within the lifetime of this project license.

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

Our CAR T cell program is known as the largest CAR T cell program outside of the USA/China with a greater number of open clinical CAR Studies then the rest of Europe combined. This department routinely shares data to the rest of our establishment, at national and international scientific symposia, in scientific journals and to patient interest groups.

**Species and numbers of animals expected to be used**

- Mice: 7000
- Rats: 200

## Predicted harms

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

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

Mice are the lowest vertebrate group in which our model systems can be applied and will be used for the majority of this license.

In general, young adult animals will be used due to the consistency of these models in this age groups. Additionally, for this project, a small number of mice will express elements of the human immune system (eg express human proteins on mouse cells or containing human immune cells). These are called humanised mice and will allow us to model human immune system in these mice so we can assess treatments for human cancer patients. For this work, a small number of young neonatal mice will be used as human stem cells can be introduced and grow better in young mice.

Moreover, a small number of adult rats will also be used as these animals are particularly useful for developing antibodies against certain tumour proteins that can't be generated in a mouse.

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

Typically cancer cells will be introduced to a mouse by injection of a small volume of fluid and cells into the vein of animals, brain or under the skin. Mice will be given a general anaesthetic for injections into the brain but are frequently kept awake for injections into the vein and under the skin. Tumours will be allowed to develop and then these mice will be treated with CAR T cells to measure response. Where possible, animals are anaesthetised (so they are not awake for the procedure) and then scanned every few days to every week in order to monitor extent of cancer. Alternatively mice may have a small blood sample taken (not more than once a week). The length of these experiments will be kept as short as possible and can vary from two weeks to several months depending on the type of cancer studied. The number a procedures will be kept to a minimum.

Animals will treated with care in accordance with national guidelines and be humanely killed at the end of experiments. In addition they will be monitored daily and observed in detail for signs of suffering. Clear thresholds are set to establish limits to an animal's suffering during this work and if an animal meets these thresholds, the animal will be humanely killed. When animals undergo certain procedures, they may need to receive general anaesthesia. They are kept warm throughout, length of anaesthesia kept to a minimum and then animals are observed till the fully recover.

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

The impact on animals can be grouped into 2 main categories

Affect of progressive cancer. Local affects of growing tumour causing pain and weight loss or compression on nearby organs. We find that haemopoietic cancers can potentially cause hind limb paralysis due to vertebral involvement with disease and subsequent spinal cord compression. Each tumour model has its own pattern (of side effects and kinetics of onset) thus it important the scientists using these models have an understanding of the typical timings of these possible tumour related side effects

Affects of the CAR T cell treatment. CAR T cell therapy can occasionally result in immune activation in human patients (called cytokine release syndrome or CRS) as well as in CAR animal models. Typically in mice, this will manifest in weight loss and reduced activity starting within 72 hours of CAR treatment. Very rarely these CAR T cells can also damage the (non tumour) tissues of the mouse called graft versus host disease. This usually occurs weeks after CAR treatment. Exact timings vary according to the tumour model and particular CAR used.

Understanding the possible impact on the animals in experiments (in terms of frequency and timings of onset) is vital to both detecting and subsequently managing these impacts to minimise the suffering of animals

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

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

Overall, 50% of mice will experience mild suffering and all rats used will experience mild suffering. about 49% of mice used in this work will experience a moderate degree of suffering, and overall ~1% of mice used in this work will experience a severe degree of suffering.

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

- Used in other projects

**A retrospective assessment of these predicted harms will be due by 18 July 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?**

CAR T cells have been hailed as the medical breakthrough of a generation but CAR T cells only work for a small number of cancers, can be associated with serious side effects or patients do not respond long term and cancer returns. We aim to develop safer and more effective CAR T cells that also work in a larger number of cancer types. This project license is a crucial part of a larger program of work which uses a range of tests in the laboratory, including computer analytical systems and detection of particular proteins and genes in order to test function of these CAR T cells against cancer. However before testing in human patients, it is necessary to test the function of these cells in mouse models of cancer. There is no replacement for analysing how these cells work in the complexity of living systems and this is necessary before these CAR T cells are brought to clinic to treat patients with cancer.

There is no replacement for analysing how these cells work in the complexity of living systems. To expand on this, in the first instance, tumour cells are not the same as cancer cells in the laboratory. In a living being, a CAR T cell needs to first get to tumour by travelling in the blood, crossing walls around blood vessels and particular organs and getting through tissue before it can kill cancer cells. This is impossible to faithfully replicate in the laboratory or by computer programmes so when designing new CAR treatments, it is important to observe that tumour cells can still be treated in animals. Further CAR T cells don’t just have to reach tumour, they also have to be able to function well at the site of tumour. For example the immune system is a complex system of many cells, organs, proteins and tissues. The immune system can prevent animals from cancer and so cancer frequently manipulates or changes the immune system to allow its own growth. As CAR T cells are immune cells themselves, these same factors that dampen down the normal immune system to allow a cancer cell to grow also make the CAR T cell work less well. So equally crucial to understanding how effective CARs will be in patients will be understanding the interaction between CARs and their surroundings and if they can still kill cancer cells in this setting. This is necessary before these CAR T cells are brought to clinic to treat patients with cancer.

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

Indeed there is an extensive laboratory program that does not involve animals and experiments requiring animals make up only a small proportion of the overall programme to develop and new and more effective CAR T cells for cancer patients. In general, the process of developing new or better CAR T cell can be typically split into 5 main steps Developing a new or safer way of targeting cancer cells. For example we decide from a literature review or new research on a new cancer target (or ‘antigen’) with CAR T cell therapy

We need to develop a molecule that is able to bind this chosen cancer target. There are several approaches for doing this. In the past we have used known molecules that bind

the cancer target of interest which does not involve animals. Alternatively, a common method is to develop particular binding proteins called antibodies to bind the cancer protein. A common and efficient way of making antibodies involves using animals such as mice or rats. However there are ways of making antibodies without using animals which we do attempt in the lab but this does not always result in antibodies which bind as well so thus result in CAR T cells which work less well. Thus it is common that antibodies need the use of animals.

We need to choose the best antibody. Step 2 usually produces a number of possible antibodies and the process of choosing the most appropriate antibody (or 'panning') involves a series of experiments in the laboratory. Binding of the candidate antibodies are sequentially tested with cancer target or cells that contain the cancer protein on its surface to progressively narrow the antibodies until the best antibody is chosen. This occurs in the laboratory and does not involve animals.

Having chosen the best antibody, there follows a series of extensive experiments in the laboratory to develop the most promising CAR T cell. Here. the selected antibody is used to form different CAR T cells (or various formats) and these CAR T cells are the tested in laboratory to make sure they kill cells that contain the tumour target or tumour cells themselves. Killing has to be demonstrated in multiple experiments and under conditions where only the best CAR T cell format will be able to kill. Further experiments will then ensure that CAR T cells don't kill once but are able to keep killing, that they can release chemicals called cytokines (which, in turn, increase CAR killing), that they are able to divide quickly and efficiently to make new, daughter CAR T cells. These series of experiments are rigorous, take at least 6 months, occurs solely in the laboratory and does not involve animals. After this step, a single CAR T cell format will be deemed the most promising. This CAR T cell will then be assessed in animal experiments

Only the most promising CAR T cells format is assessed in animal models. This is a necessary part of ensuring CAR T cell function before proceeding to assessment of the CAR T cells in patients

As can be seen above, experiments requiring animals is a small but vital part of the process to develop new, better or safer CAR T cells for patients with cancer (possibly in Step 2 and Step 5). Non-animal alternatives are being developed.

For example, antibody generation in animals (Step 2) don't always work and there are other methods of developing antibodies without using animals. However, in turn these animal free methods are also not always successful, until these methods are improved, we may need to use animal for this work. As a lab, we will continue to actively seek non animal methods to generate antibodies and look forward to the time when animals are no longer used for this work

I have described in the section before that animals are required (For Step 5) because it is impossible to replicate the complexity of living organisms in the laboratory or by computer programming. However laboratory methods are being developed to approximate tumours in animals are patients (for example special methods to grow cancer cells in the laboratory called organoids). Thus we will attempt to use these emerging methods as much as possible rather than animals

**Why were they not suitable?**

As mentioned before, animal free methods to generate antibodies are also not always successful, until these methods are improved, we may need to use animal for this work. As a lab, we will continue to actively seek non animal methods to generate antibodies and look forward to the time when animals are no longer used for this work
Similarly, there are no methods to faithfully replicate the tumour conditions in the laboratory thus animals remain part of this laboratory programme.

**A retrospective assessment of replacement will be due by 18 July 2026**

The PPL holder will be required to disclose:

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

## Reduction

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

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

These numbers have been based on our own past and ongoing work on these mouse models as well as published data. Statistical tests have then been used to calculate the minimum number of mice needed to demonstrate a true difference between treated and untreated mice in experiments

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

We plan to use a variety of techniques to reduce the number of animals and will keep our work in adherence to NC3R's ARRIVE guidelines. Initial pilot experiments will be performed to establish parameters needed to determine cohort sizes (eg expected change in outcome such as CAR persistence or survival, signal to noise ratio). Size calculations will be performed using statistical packages available from Select Statistical Services or G*Power.

The sizes of experimental groups and the number of repeated experiments will be kept as small as possible while ensuring that results are real, reproducible and of clear biological significance. Further, we will have several readouts from every sample taken and each mouse so that we can maximize the information we get from every experiment.

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

The CAR T cell department do have extensive experience in running the models described which means that experiments can be run efficiently. Where possible, breeding of mice will be managed at a centralised facility ensuring that the best breeding methods are consistently used. Thirdly the latest published works will be used to make judgement on

design for new experiments. This will be followed on small or pilot experiments on small numbers of animals so that we will be better placed to design larger more informative experiments, which are both adequately powered and reproducible, by size calculations described above.

**A retrospective assessment of reduction will be due by 18 July 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.**

These models aim to understand how CAR T cells will behave in cancer patients. To study how CAR T cells can clear tumour, we will introduce cancer to mice by injecting tumour cells into the vein, under the skin or in the brain. Alternatively we will use models that develop cancer on introduction of a particular drug or we may breed mice that spontaneously develop cancer. These animals with cancer will then be injected with a dose of CAR T cells by vein as treatment.

In the models described here in addition to close observation and where possible, mice are monitored by small blood tests, or scans to be able to track tumour growth before the mice become unwell. These measures aim to continually track tumour response and do so before the animals experience pain, suffering or distress. The experiments can therefore be completed before the animals become unwell and also allows the most amount of data to be obtained from each mouse. These mice will be observed, a small number of blood tests taken or mice are scanned so we can track tumour growth following treatment. Secondly, CAR T cell therapy in humans can result in severe side effects that can be life threatening. CAR T cells can inadvertently target normal human tissue or cause an illness called cytokine release syndrome. We plan to use a number of humanised mouse models to develop CAR T cells that do not inadvertently target normal human cells or cause CRS. Here, young mice are injected with a small amount of human stem cells in the liver or older mice undergo surgery to implant a small piece of human tissue. Tumour is then introduced into these mice before they receive CAR T cells.

In the models described here mice are monitored using blood test or by labelling the transferred cells allowing these cells to be monitored by scanning allowing the transferred cells to be monitored in real time. Once again this maximizes the information made available from each mouse and these models are not reliant on animals becoming unwell.

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

There are similarities between the physiological systems of mice and humans, including immune systems. For example different types of immune cells exist in both mice and human eg B and T lymphoid cells and subsets of these cell types can be identified in both species. Further, the function and anatomy of major organs in both species are similar. These are important for two main reasons. Firstly, it means that mice serve as a good model for human disease including cancers and are able to model both how cancer develops (also called disease evolution) as well as response to treatment. Secondly, we also have an interest in the development of immunecompetent CAR/cancer models. These are models in animals with a normal immune system which then have a mice version of the cancer which we then treat with mice CAR T cells because these models should best replicate cancer and CAR T cell treatment in patients. Wiith the ultimate aim of developing CAR T cells that work better in patients, we aim to use these realistic models to study the interaction between CAR T cells and other immune cells/proteins/organs in patients. The similarity between human and mice immune systems allow findings in these models to be correlated to humans.

Therefore mice are the lowest vertebrate group in which our model systems can be applied. Animals at a more immature life stage do not have a fully mature immune system so are therefore a less suitable models for therapeutic T cells. Similarly interactions between the CAR T cells and host immune system or tumour need to be studied over time and in living animals so therefore cannot be studied in terminally anaesthetised mice.

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

The studies will be designed to use the simplest and least harmful methods possible to obtain the data required for completion while also reducing suffering to test animals. The two major causes of suffering  is due to cancer progression or CAR related toxicity. Thus procedures to improve welfare of animals are largely focused on monitoring tumour growth and CAR toxicity

All experiments will have predetermined frequency of monitoring, method of monitoring and also limits for the experiment

These parameters will be under constant review. Eg in the event of a breach of license limits, a root cause analysis will be performed, SC18 form completed and discussion with NACWO to refine experimental procedures from that point on.

Methods to optimally monitor tumour progression or CAR toxicity and their toxicity will also be constantly under review. These methods include:

- Optimised flow cytometry panels
- Assays for particular serum markers (by ELISA or serum electrophoresis)
- Imaging methods such as MRI or CT scans or labelling tumour or CAR T cells with (bioluminescent) proteins that fluoresce and can be detected by scanning.

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

Protocols are published for tumour models, CAR monitoring, specific toxicities. However as CAR/myeloma models have been and continue to be developed for this project, best practice guidance is then refined for individual models. For example, in the lab we frequently use a quarter of recommended venesection volume for CAR monitoring compared to published standards PMID: 35152996) and published scores (eg GVHD PMID: 31180340) are continuously reviewed and compared to the existing clinical observational score on this license and a decision made as to if a new scoring system has to be added to the license balanced against potential confusion caused by multiple different scores across the license.

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

The Project Holder and licensees take responsibility for keeping abreast of developments through conferences, current knowledge of published literature and will benefit from the network of institutional support available through our Biological Services Units who contact license/project holders with developments and offer regular update sessions.

**A retrospective assessment of refinement will be due by 18 July 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?

# 34. Studying the stem cell origins of cancer initiation and progression

**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**

cancer, stem cells, metastasis, regeneration, treatment

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

## Retrospective assessment

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

**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 primary objective of this project licence is to better understand the impact of intrinsic (genetic) and extrinsic (environmental) factors that transform tissue resident stem cells leading to cancer initiation and progression and overall increased life-time cancer risk.

**A retrospective assessment of these aims will be due by 14 July 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?**

One in two adults will get cancer in their lifetime imposing on society enormous human and fiscal costs. This statistic does not fare better worldwide and it is estimated that cancer incidence rates will continue to increase over the next two decades. Understanding the earliest events that drive normal stem cells to become malignant (cancer initiation) is one of the most important challenges facing cancer research. Better understanding of this process could lead to new approaches for early cancer detection and diagnosis, which would markedly reduce cancer incidence and/or begin treatments earlier to prevent progression. Importantly, understanding how these are similar or differ among cancers will provide insights on the fundamental basis for the origins of disease agnostic of cancer type.

It is critical to address these challenges in cancer research for the following reasons:

- Cancer incidence and deaths are projected to increase over the next two to three decades
- The global cost of cancer is estimated at $1.1 trillion/year making it an incredibly expensive disease to treat (https://www.who.int/news-room/fact-sheets/detail/cancer)
- >90% of solid cancer-related deaths are attributed to metastatic disease
- The impact on patients and their families is devastating

We propose in this licence to utilise a variety of well-defined stem cell driven tumour initiating models to further our knowledge on the mechanisms involved in initiation and progression of each tumour type. Our project holds great promise to make fundamental and much needed progress in advancing understanding of the origins and biology of a great variety of adult cancers.

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

The biology of cancer-risk and metastatic disease are active areas of research and it is crucial to understand the basis for these in order to create and develop novel therapeutic approaches for cancer and metastatic spread prevention.

Understanding the basis for cancer risk, and how the process of development prevents this process will generate four outputs at the end of the project.

The field of cancer risk/stem cell biology will move forward in a such a way that a sub-field of research in this area will be created. The concept of cancer risk being a non-random process is a brand-new idea and this will open many more avenues of research within this area

By identifying how neonates are protected from getting cancer, we can re-activate this mechanism in adults. We hypothesise that re-activating this protective mechanism in adults will reduce cancer risk and incidence, providing a novel approach to cancer prevention

A comprehensive atlas of ageing and resource for stem cell biology will be made available for the scientific community

The experiments we propose for understanding the basic principles governing cellular metastasis in normality and cancer will provide new information on potential treatment strategies for metastatic disease by removing the context of cancer to focus on the core elements associated with cell trafficking

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

In the short-term (2-3 years), we will regularly keep the scientific community updated on the progress of our research in the forms of publication and presentations at conferences both local and international platforms. We will also engage the public through events held at our institute. Additionally, the project proposed will provide a foundation for training of master students and/or summer students. The scientific community will benefit from our work through new knowledge gained in the field as well as resources made available to the community (sequencing data etc.).

The long-term aspect of these projects has tremendous impact in society. Cancer is a devastating disease, both for patients and their families. The spectrum of the cancer experience is very unpleasant and given that cancer incidence is projected to rise, measures to eradicate this disease are desperately needed. Understanding how cancer risk is dictated will, in the long term, provide a very powerful preventative approach for cancer in humans. By understanding the factors that govern cancer risk, we can target these factors in adults and use this as a viable approach to preventing cancer formation in the first place. This approach is extremely powerful and has implications in society beyond cancer prevention alone. By eradicating or significantly reducing cancer incidence in society, the quality of life for adults is massively improved, especially as cancer is a disease many elderly people are burdened with. This would subsequently reduce the burden on health organisations from having to treat cancer in the first place. Given that we will likely find a protective mechanism, this could also be used as a potential biomarker in adults to identify patients that will likely have cancer in the future, again, improving outcomes for patients and society as a whole.

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

We will regularly publish our work to make our data and findings available to the scientific communities as soon as possible. Resources generated form the experiments proposed (e.g. mouse models, sequencing data, images, etc…) will be made available in appropriate databases, such as GEO for sequencing data, in order to make it freely available for researchers to use. Moreover, the universal atlas of aging across organs in stem cell populations will provide a new resource that can be used to interrogate additional scientific questions on the more global scale of the aging process at a cellular level. Open access to this resource will prevent further and unnecessary animal research relating to aging as the resources are already available.

**Species and numbers of animals expected to be used**

- Mice: 17750

## Predicted harms

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

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

The overall aim of these projects is to better understand cancer biology, from initiation through progression. Mice represent an ideal model for addressing these aims. Non-mammalian models do not share such similarities and are unable to reproduce many of the biological and physiological features of human cancers. In vitro systems cannot currently replicate the complexities of the mammalian organ systems which include studying cancer risk and metastasis. The scientific community has a range of techniques that enable manipulation of the mouse genome, allowing access to many transgenic/knockin/knock-out mouse models with which we can answer specific key questions regarding tumour biology. Each stage has been specifically selected as those most appropriate for the study. The choice of life stages are summarised as follows:

- Characterising a spectrum of cancer risk spanning mouse ages, from birth to old age, allows us to interrogate the unique inherent risk of cancer associated with aging in a mammalian system, which will give us important insights into the human risk leading to testable hypotheses.

- Mouse tumour models recapitulate human cancer, in space and relative time and so mice at varying ages, depending on the tumour model are required to accurately capture tumour initiation and metastasis.

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

Many animals in this project will develop tumours (e.g. gastrointestinal tumours) and/or be exposed to organ damaging agents. In order to study the relationships of factors in cancer risk, initiation and progression.

- some mice will develop tumours (either spontaneously or following administration of transducing substances or following implantation of genetically modified cells), may be exposed to organ damaging agents and aged to monitor for tumour formation. During this period animals may:

- undergo multiple rounds of non-invasive imaging method(s), which requires light anaesthesia and administration of a substance to enable visualisation of the tumour as it grows have blood withdrawn multiple times throughout life to monitor disease progression

- receive newly developed, but thoroughly tested, drugs or agents delivered by a variety of methods including injections, oral gavage, or in drinking water

- all animals used in this project will be killed using a schedule 1 method following the study.

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

Throughout the experimental procedures carried out in the various protocols, animals will experience the following adverse effects:

Transducing agents: Administration of substances via intraperitoneal or intravenous injection, or oral gavage will cause recurrent (daily or weekly), transient (10-15 minutes) discomfort and distress, due to the restraint and procedure required. Repeated intraperitoneal/intramuscular injections can result in tissue damage in rare (<2%) cases. The majority of cases will result in transient and mild discomfort.

Administration of perturbing agents: A perturbing agent is an agent that can modify protein function or gene expression, for example, ion channel inhibitors. Administration of substances may lead to neurological complications (e.g. mild tremors), respiratory distress, lethargy, digestive disturbances and altered cardiac function.

Organ damage: Adverse effects as a result of organ damage is dependent on the agent used and the organ targeted. Animals will likely experience localised adverse effects including respiratory distress, abdominal discomfort and skin ulcerations depending on the agent used. Additional adverse effects as a result of organ damage may include lethargy, hunched posture, lack of grooming and physical discomfort which may include signs of pain and weight loss up to 20%.

Tumour formation: may lead to extensive abdominal distention, body-condition deterioration, digestive disturbances, respiratory distress, or neurological/behavioural abnormalities, lethargy and transient weight loss up to 15%.

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

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

The majority of animals used within this project (~70%) will experience a moderate level of severity. The remaining animals (~20%) will be expected to be in the mild or subthreshold category.

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

Killed

**A retrospective assessment of these predicted harms will be due by 14 July 2026**

The PPL holder will be required to disclose:

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

## Replacement

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

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

Although *in vitro* cell culture methods can be used to test the transformative effect of gene mutations on particular cell types, this system cannot recapitulate the complex microenvironment that exists in tissues and organ systems. Our scientific approach requires the use of cell types that are susceptible to cancer, at specific time points during development and animals remain the most informative model, as they recapitulate the diversity of cell populations, complexity of human development and the crosstalk between tumour and stromal cells in human cancers. Additionally, to fully understand how disease not only initiates but progresses and how this progression occurs through various routes in the body requires the use of living animals that best mimic the human conditions

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

Our approach requires the use of specific cancer-susceptible cell types. While *in vitro* models have shown to be successful for studying cell line specific biology, they cannot fully recapitulate the complex physiological environment that governs cancer risk, formation and metastatic spread.

**Why were they not suitable?**

Studying cancer risk, tumour initiation and progression via metastatic disease is currently only possible by using live animals that fully recapitulate the complexities and diverse cell populations that contribute to these processes.

**A retrospective assessment of replacement will be due by 14 July 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 use of *in vivo* mouse model experiments has a careful statistical design that is aimed at minimising the use of animals while ensuring robust and meaningful statistical end points. These animal numbers are selected based on our extensive experience with these mouse models.

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

In designing the experiments described within this project we aimed as much as reasonably practicable to reduce, replace and refine our animal models. Where mouse model experiments are required, we have used a careful experimental design that allows us to minimise the use of animals, whilst achieving the maximum amount of data and

maintaining reliable statistical end points. For example, when organ harvesting, we will take as many organs as possible to maximise the data acquired from individual mice, reducing animals needed to successfully achieve our aims.

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

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

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

By employing standardised operating procedures for our experimental design, particularly in our novel treatment studies, we can use data from control animals in one study, as controls in another (within the same tumour type) and thereby reducing the number of animals required in this project. Where needed, pilot studies will be performed which will inform the design of subsequent studies, potentially enabling the reduction of numbers. During our previous project licence, we were able to refine our breeding strategies that will be directly transferable to this project licence. This has resulted in a reduced number of mice required for breeding. Our results thus far have been analysed using advanced computational methods and we have very clear ideas about how to proceed with future mouse experiments which would minimise the number of animals required.

**A retrospective assessment of reduction will be due by 14 July 2026**

The PPL holder will be required to disclose:

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

## Refinement

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

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

Mice are the only species that are employed in our protocols. In particular, we will use genetically engineered mouse models (GEMMs) that replicate multiple types of human

cancers (e.g. gastrointestinal). These strains have been specifically generated to carry the genetic mutations that lead to tumour formation in the human diseases. In this project we will use these strains to explore the biology of the disease by inducing tumour formation using a variety of methods including conventional breeding or injection of agents. Animals in these studies will be maintained under normal conditions until a specified time, at which point material will be collected and analysed as previously described.

Organ damage models will be used to understand how environmental factors impact life-long cancer risk. We will use damaging agents to study the regeneration of the liver (DDC diet) ,colon (colitis) ), kidney (nephrotoxic), and skin (radiation, physical)with and without known tumour causing mutations. The organ damage models can induce rapid changes that are able to be repaired just as rapidly on the order of days.

Implantation of genetically modified cells under the skin (subcutaneous), into a blood vessel (intravenous), into the abdomen (intraperitoneal)), orthotopic sites (gastric submucosa, mammary fat pads) will be carried out in immune compromised (NRG, Nu/nu) and immune competent (C57Bl/6J and our own genetically engineered animals) mice.
We will minimise suffering by adhering to the best practice guidance, currently the NCRI guidelines for the welfare and use of animals in cancer research. Every protocol proposed in this licence is the most refined for the purpose and designed to cause the minimum distress and suffering to the animals.

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

Mice are the most relevant species with the least sentience that we can use to carry out the research proposed in this project. Their short lifespan (approximately 2 years) allows for studies in which disease progression can be monitored throughout development to humane endpoint, mimicking far closer the disease progression in humans. Further to this, the short gestation time (~3 weeks), extensive published knowledge and the array of techniques that enable manipulation of the mouse genome, allows us access to genetically modified animals in which we can explore the effects of genes on normal development and tumour formation in a species where the process is similar to that in humans. Less sentient species do not follow this same developmental program. Non-animal models cannot recapitulate the complex context of tissues in which cancers develop. These features are critical for understanding the impact of potential novel therapeutic approaches.

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

Our procedures will be regularly assessed throughout the project in order to ensure that they are being performed in the most refined manner. Refinements will be routinely made over the course of the project through constant advice from veterinary and husbandry staff, as well as through regular liaison with clinical practitioners. If needed amendments to the licence will be applied for, for example, if new procedures are needed to improve the welfare of animals.

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

Work in this licence will be undertaken in accordance with the principles set out in the Guidelines for the welfare and use of animals in cancer research: British Journal of Cancer

(2010):102: 1555-1577 and the guidelines published by the NC3Rs, which are updated regularly with the best current practice. Blood sampling will be performed as recommended in the NC3Rs guidelines.

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

We currently follow updates provided by the National Centre for the 3Rs through receipt of electronic and printed media and through regular attendance at the AWERB committee at the institute.

Throughout this project we will continue to communicate constantly with the veterinary, NACWOs and husbandry staff, receive updates from the NC3Rs and will attend NC3R and scientific conferences. These approaches will allow us to be aware of the most recent advances in the field and will enable us to implement these into our research in a timely manner.

**A retrospective assessment of refinement will be due by 14 July 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?