



Home Office

NON-TECHNICAL SUMMARY

The role of the immune system in cardiovascular disease

Project duration

5 years 0 months

Project purpose

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

Key words

cardiovascular disease, immune system, pathophysiology, inflammation, therapy

Animal types

Life stages

Mice

adult, neonate, juvenile, embryo, pregnant

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- ◆ Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

Diseases of the heart and the circulatory system are mostly due to blockage of blood vessels by a process called atherosclerosis (fatty deposits), and are responsible for heart attack, heart failure, and stroke. We want to understand why our immune defence system goes awry and contributes to the development and progression of these diseases, with the aim to find appropriate treatments.

A retrospective assessment of these aims will be due by 06 January 2027

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?

Diseases of the heart and the circulatory system are still one of the major causes of disability and death worldwide. There's increasing evidence that our immune defence system plays an important role in heart and blood vessel diseases. However, our understanding of how this happens is still limited, and this has prevented scientists from finding new effective treatments. We believe that an improved understanding of the role of the immune system in cardiovascular disease may transform the way we treat patients with (or at risk of) the disease.

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

The present research will lead to a better understanding of how the immune system reacts against fatty deposits in our arteries, and how it controls cardiac repair after injury. The results are expected to lead to more effective strategies to combat atherosclerosis (the blockage of arteries due to fatty deposits) and its complications (heart attack, heart failure). We also expect that the work will substantially enhance our understanding of aneurysm (bulging or ballooning of arteries) growth and rupture, and will identify critical targets for treatment. Our results will be published in peer-reviewed scientific journals, and may lead to the filing of patent applications.

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

Atherosclerosis is responsible for the vast majority of cardiovascular diseases (CVD). CVD, including aortic aneurysm, are the largest contributors to disability and death, in Europe and worldwide. There are around 7.6 million people living with heart and circulatory diseases in the UK. CVD cause more than a quarter of all deaths in the UK— an average of 450 deaths each day or one every three minutes. CVD markedly impact on both quality and length of life of patients. CVD carry an important socio-economic burden. CVD's cost to the UK economy (including premature death, disability and informal costs) is estimated to be £19 billion each year (BHF statistics compendium, <https://www.bhf.org.uk/what-we-do/our-research/heart-statistics/heart-statistics-publications/cardiovascular-disease-statistics-2020>).

Direct likely immediate benefits include:

- 1) Development of new and more relevant ways of investigating aspects of CVD in rodents that can be used by other researchers. For example, we are aiming to develop better ways to study cardiac cachexia (weight loss associated with heart failure, which bodes for increased disability and risk of death) to be able to better understand the disease in humans;
- 2) Better understanding of the pathophysiology of atherosclerosis (blockage of arteries), myocardial infarction (heart attack) and vascular aneurysm (ballooning of the blood vessels);
- 3) Identification of new targets to better treat patients with CVD. Our results will therefore benefit the scientific community through the provision of important new knowledge about mechanisms of disease.

Indirect long-term benefits include:

- 1) Better understanding of the role of specific cells of the immune system in CVD;
- 2) Develop new ways to better diagnose patients with CVD (identify individuals with the disease and quantify the risk of disease progression);
- 3) Develop better treatments for patients with atherosclerosis, myocardial infarction, heart failure, and aneurysm.

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

We work in collaboration with an extensive network of doctors and scientists. My group is part of several national and international research networks. I am co-Editor, Associate Editor or Consulting Editor of many highly respected international peer-reviewed scientific journals, and I am member of highly respected scientific societies worldwide. Our results, whether successful or not, will be presented and discussed at international scientific meetings, published in peer-reviewed scientific journals, and disseminated to the general public.

Species and numbers of animals expected to be used

- ♦ Mice: 44300

Predicted harms

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

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

For the most part of our work, we are not studying congenital (inherited or present from birth) heart diseases. Therefore, most of our work will be conducted in adult animals.

We have chosen mice because 1) they are widely validated animal models of heart and circulatory diseases, 2) a large number of genetically-altered mice have been developed and are available to the scientific community, which makes possible the study of the role of specific gene defects in the development and progression of heart and circulatory diseases.

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

A typical animal will be put on normal or high fat diet for 6 to 12 weeks, will undergo blood sampling (potentially after fasting up to 16h) less than 3 times during the duration of the experiment, will regularly be monitored clinically, and killed while under terminal anaesthesia (a state of induced unconsciousness) at the end of the experiment. The animal will not recover from the deep unconscious state before being killed.

Another typical example is an animal undergoing a procedure that will cause heart vessel injury, being regularly monitored clinically and through non-harmful imaging to assess disease development and progression, and killed without recovering from terminal anaesthesia at the end of the experiment.

In most cases, the mean number of regulated procedures in a given experiment is less than 5.

A worst case scenario would include the above (from a typical experiment) with 1 to 3 additional procedures, which would include for example, whole body irradiation and bone marrow reconstitution (which is undertaken to better understand the specific contribution of the immune defence system to the disease process), injection (for example in the veins) of gene inducing or disease modifying agents/substances to modulate disease state and the immune system, or single housing for a few days (with or without food restriction) to assess food intake and how the body burns and uses food components.

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

There are adverse effects related to the obligatory procedures in each experiment. These may include pain after a surgical procedure (animal may have hunched posture, may reduce its activity and become immobile), or pain due to heart attack or imminent aneurysm rupture, or shortness of breath or weight loss associated with heart failure.

In most cases, the estimated duration of these effects is less than a few hours, and animals are monitored regularly so we can avoid or treat the adverse effects, e.g., treating pain by use of analgesics

(pain killers), or terminating the experiment preventively (humanely killed) before adverse effects approach humane endpoints.

In some experiments, severe levels of suffering may be reached, and they are mostly a consequence of the disease process being studied, e.g., heart failure with severe shortness of breath, or heart failure associated with severe weight loss. In this case, these "adverse" effects may be allowed to last up to 48 hours, because our aim is precisely to understand how and why these effects occur, and be able to find treatments to prevent them, first in animals, and hopefully in patients. In all such cases, animals are very closely monitored (several times a day), so they can be humanely killed before reaching the maximum severity level allowed on the experiment.

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

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

Mice: Mild (57.6%), Moderate (31.8%), Severe (10.6%).

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 06 January 2027

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 need to use animals is due to the lack of reliable alternative ways (e.g., experiments done in test-tubes) that would allow a correct understanding of the diseases we are studying in this project. In particular, no test-tube assay will correctly predict the occurrence of heart failure after a heart attack, the occurrence of weight loss after heart failure, or the rupture of a great artery. This is why we need to use living animals to understand the complexity of the disease process and test the potential effect of new treatments.

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

During the last few years, we have developed the use of human cells that can be manipulated in a dish, to reproduce some aspects of the disease process that occurs in the living animal, with the aim of replacing the use of animals. This has allowed us to replace and avoid the use of animals in certain circumstances. We will continue to test other non-animal strategies with the aim to replace the use of animals.

Why were they not suitable?

Some alternatives have proved to be suitable.

We have also tried to use, in collaboration with other investigators, other animal species that are not protected by law, like immature pre-independent feeding zebrafish. However, we found that these animals were not suited to study the complex disease processes we are interested in (e.g., blockage of arteries).

Other alternatives are just not feasible. In particular, no in-dish assay will correctly predict the occurrence of heart failure after a heart attack, the occurrence of weight loss after heart failure, or the rupture of a great artery.

A retrospective assessment of replacement will be due by 06 January 2027

The PPL holder will be required to disclose:

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

Reduction

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

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

We have been using mice in our cardiovascular research for 25 years. We therefore have previous data and experience on which to determine the numbers of animals we are likely to require for this programme of research. We will also use statistics and consult expert biostatisticians when necessary.

The total number of mice for this project may seem relatively high. However, my laboratory is relatively big with 15 to 18 researchers (both juniors and seniors) involved in experiments on animals.

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

We follow the PREPARE guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and the CAMARADES/NC3Rs systematic review facility recommendations for the design and analysis of our experiments. When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative.

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

Breeding strategies are designed to produce the correct number of animals with the correct genetic make-up (e.g., ensure that the gene or set of genes we have modified are correctly expressed).

When there's little information available, pilot studies are conducted in order to improve our estimation of the numbers of animals required to detect a significant effect.

We always aim to maximise the information that can be recovered from a single animal. For example, the same animal may undergo repeated non-harmful imaging over the course of an experiment (in order to assess the progression of the disease process) rather than killing different animals for each time-point in an experiment. When animals are killed at the end of the experiment, samples are collected from multiple organs to assess the effect of candidate gene mis-expression in multiple tissues.

A retrospective assessment of reduction will be due by 06 January 2027

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 widely validated ways to investigate cardiovascular diseases, in particular experiments in living animals that reproduce the disease process of atherosclerosis (blockage of arteries due to fat deposits), myocardial infarction (heart attack), heart failure, and aneurysm (bulging of arteries) seen in patients. Animals used here are mice because this species has been shown to be highly suitable to study these disease processes. Our expertise in these animal procedures is internationally recognised. We follow the most updated recommendations for animal experimentation. Animals are housed according to the best recommendations in appropriate and enriched environments. By performing pilot studies and choosing well established experimentation procedures based on extensive previous experience (more

than 25 years), we minimise the unknown effects on the mice and subsequently pain, distress and suffering.

Some experimental procedures may be associated with a severe level of suffering. This is inherent to the disease process being studied (e.g., heart failure with severe weight loss, rupture of a blood vessel due to aneurysm), which also causes suffering and distress in patients with these forms of the disease (and is the reason why we are studying the process with the aim to find appropriate treatments). We monitor these animals very closely to pre-empt signs of pain or distress and to be able to terminate the experiment based on appropriate humane endpoints that minimise animal suffering.

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

We have tried, in collaboration with other colleagues, to use zebrafish to study aspects of atherosclerosis (blockage of arteries). Unfortunately, it appears that this type of animal is not suitable because it does not develop the advanced stages of the disease (which are responsible for the suffering and sometimes the death of patients with atherosclerosis).

In a few cases, we will perform the experiment under an anaesthetised state (total unconsciousness of the animal) to address some aspects of the disease process.

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

The number of techniques and surgical procedures used in each experiment is limited in order to reduce the harms caused to each animal while still obtaining scientifically sound information.

We very frequently monitor animal behaviour and well being to detect signs of pain and distress at an early stage.

When necessary, we familiarise animals with changes in their environment. As an example, when there's a need to assess individual food intake and how each animal burns and uses food components, animals must be singly housed in specific cages for up to 10 days. Single housing may be associated with some stress and some animals may show aggressive behaviour after re-housing with littermates. As a refinement control measure, pre-exposure to soiled bedding for several days prior to regrouping may be used in order to reduce aggression. Our refinement measures in this setting agree with the work and recommendations of Jane Hurst (<https://www.nc3rs.org.uk/sites/default/files/documents/NC3RsarticleJaneHurst%20making%20sense%20of%20scents.pdf>)

We always aim to maximise the information that can be recovered from a single animal. For example, the same animal may undergo repeated non-harmful imaging over the course of an experiment (in order to assess the progression of the disease process) rather than use different animals for each time-point. When animals are killed at the end of the experiment, samples are collected from multiple organs to assess the effect of candidate gene mis-expression in multiple tissues.

We have developed and refined the procedures we used in mice to enable us to use this type of animal to explore how aspects of aneurysm (i.e. ballooning of a blood vessel) occur. This has been achieved

mainly by using a new simplified surgical procedure associated with reduced vessel injury and much shorter duration of surgery. We will pursue such important efforts to improve our use of animals to ensure we minimise as far as is possible animal distress and suffering.

We will use established observation methods for monitoring animals, e.g., scoring sheets and grimace scales (doi:10.1038/nmeth.1455) to reduce suffering and use of pain relieving medication when appropriate.

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

We use widely established experimental procedures, and follow accepted guidelines, including ARRIVE, PREPARE, LASA Aseptic surgery guidance (2017), and NC3Rs recommendations, to ensure optimal planning, reporting and refinement of animal experiments.

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

We are at the forefront of research in cardiovascular medicine. We follow the ARRIVE guidelines (2020, 2nd edition), and other developments and advances in the 3Rs (<https://nc3rs.org.uk/3rs-advice-project-licence-applicants-refinement>), including Norecopa platform (<https://norecopa.no/alternatives/the-three-rs>) and the Danish 3R-Centre (<https://en.3rcenter.dk/>) with the aim to implement them swiftly.

A retrospective assessment of refinement will be due by 06 January 2027

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?