Home Office

NON-TECHNICAL SUMMARY

Inflammation 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, heart failure, inflammation, therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal
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?

Past studies have shown that the cell skeletal structures are involved in regulating cardiovascular disease (CVD) progression through mediating the inflammatory response. To identify and validate the potential relevant therapeutic targets, we will investigate the role of associated mechanisms in inflammation, particularly in the setting of CVD.

A retrospective assessment of these aims will be due by 23 February 2030

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?

CVDs are the leading cause of death globally. CVDs cause around 1 in 3 deaths globally, an average of 56,000 people each day or one death every 1.5 seconds. Within CVDs, heart failure (HF) is a global pandemic affecting at least 26 million people worldwide, and the number is likely to increase due to an ageing population. HF is a medical condition in which the heart has a reduced ability to pump blood from its chambers. Despite the availability of some therapies, the prognosis of patients with HF remains poor. Therefore, investigating the new mechanisms for regulating the disease development of CVDs and developing treatments to improve outcomes of patients with HF are in high demand.

Previous studies have shown that cell skeletal structures are involved in regulating inflammatory output and the progression of CVDs including HF after heart attack. Further understanding the role of cell skeletal structures in regulating inflammation and cardiovascular cell function may help us to find treatment plans for relevant heart diseases.

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

1. The project will provide new knowledge in understanding the cell skeletal structures -associated roles in regulating inflammation and heart diseases.

2. Current treatments to increase heart pumping function in treating heart failure are deemed unsafe due to the increased death rate which is associated with treatments. The project may provide new treatment targets and new therapeutic strategies for improving heart function in treating heart failure.

3. Diastolic heart failure is a condition where the main pumping chamber of the heart (left ventricle) becomes stiff and unable to fill properly with blood during the relaxation phase of heart contraction, therefore affecting the amount of blood pumped out to the body. This type of HF is strongly associated with inflammation. The project will help us understand the roles of cell skeletal structures in this type of heart failure. It may provide new treatments for this disease.

4. The results of this project will be published in peer-reviewed journals and presented at relevant conferences. This project may lead to patent applications and provide important pre-clinical information for translation into humans.

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

There are around 7.6 million people living with CVDs and around 920,000 people living with HF in the UK. CVDs significantly reduce both the quality and duration of life, accounting for over 25% of all UK deaths – equivalent to 450 daily fatalities or one every three minutes. CVDs put a substantial social and economic burden on the UK (Data from BHF statistics compendium).

In the short term, the impact of our outputs will include:

1. The knowledge gained from our project will benefit a broad medical and scientific community including cardiology and beyond. Cell skeletons are structural networks within the cells and play important roles in different cellular processes. The dependent mechanism in regulating inflammation could also be used in understanding other inflammatory diseases/conditions beyond inflammatory CVDs.

2. The medical community and pharmaceutical companies will benefit from our outputs to develop new treatments for relevant cardiovascular diseases and inflammatory diseases.

3. The techniques further developed in this project (for instance different models of heart failure) may help other researchers in this field and therefore will refine or reduce mice usage in their future experiments.

In the long term, the impact of our outputs will include:

1. The novel treatments identified in this project may provide proof-of-concept evidence for future clinical trials and eventually provide safe and effective treatments for patients with heart failure and other inflammatory diseases.

2. The knowledge gained from this project may help provide detailed information to aid in the diagnosis of heart diseases with accuracy to match the relevant treatments.

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

Our previous and present projects are based on wide collaboration with different research groups worldwide. We aim to publish our results in scientific open-access journals with high impact. We will present our findings at international conferences and disseminate the new findings to the public. We'll also aim to find ways to communicate or publish unsuccessful results through conferences and open-access platforms. Our group will use the Equity, Diversity, and Inclusion (EDI) approach to involve suitable researchers from different backgrounds in the projects to maximize the output.

Species and numbers of animals expected to be used

- Mice: 15300
- Rats: 300

Predicted harms

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

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

Our experiments will be conducted in rodents. We use the mouse model in our project for the following reasons: 1). Mice and humans share a very similar set of genes. On average, the protein-coding regions of the mouse and human genomes are 85% identical; some genes are nearly 99% identical. In addition, because of the high breeding rate, the mouse model is a useful genetic model to address human biological problems. 2). There are well-established methods for mouse handling procedures. 3). The mouse models of different cardiovascular diseases including myocardial infarction (heart attack), heart failure (heart doesn't pump enough blood for the body's needs), and atherosclerosis (blockage of arteries) have been well-established and widely validated.

Adult rats will be used in heart attack models to screen effective therapeutic strategies. In some cases, rats can be advantageous due to their larger blood volume and ease of administration, allowing for more accurate dosing and monitoring of drug effects. Because of their physiological similarities to humans and their longer lifespan, results from rat models are closely translatable to human diseases. This study will help to provide essential preclinical evidence before the human clinical trial.

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

In one disease model, the animal will undergo a surgical procedure that will cause heart vessel blockage. This will be induced through one-hour surgery by creating small chest windows to access the heart and blocking a coronary artery with a single stitch. After surgery, the animals undergo regular monitoring and non-invasive imaging under anaesthesia to track disease progression. Painkillers will be applied to these animals which undergo surgery to reduce discomfort and moderate to severe pain. They will be humanely sacrificed either at an early stage (within 3 days) or a late stage (3 to 6 months) under terminal anaesthesia (a state of induced unconsciousness) to minimize suffering.

In another disease model, the animal will experience changes in food (high-fat food) and/or drinking water (chemicals inducing heart disease administered in water) lasting 5 to 15 weeks, expected to be non-distressing. Some animals may need to be housed individually in cages for short periods (48-96 hours) after receiving energy sources such as carbohydrates, insulin, and lipids. This allows for accurate measurement of food and water intake as well as urine and feces output. At the end of the experiment, animals will be humanely sacrificed under terminal anaesthesia.

Additional procedures may be included in some experiments. For example, administration of substances (e.g. treatment drug) by injection using common methods such as in the veins, under the skin, or in the abdominal cavity. Where administration is required for prolonged periods, animals will be surgically implanted with slow-release devices such as a mini-pump under anaesthesia. These animals will experience some discomfort after surgery and some mild to moderate pain which will be treated with painkillers. Some animals will experience mild and transient discomfort from blood sampling. In another situation, animals will be exposed to radiation and have their bone marrow replaced to study how the immune system affects diseases.

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

The possible adverse effects are listed as follows:

1 After the procedure that leads to reduced heart function, we expect to observe potential signs of heart failure in animals. These signs include a hunched posture, difficult breathing, decreased activity, possible immobility, signs of distress resembling a heart attack, and weight loss associated with heart failure.

These adverse effects primarily result from the development of these disease models. In most cases, they typically last for only a few hours. In certain instances, some effects may extend up to 48 hours to 1 week. All animals are subject to regular monitoring to proactively identify and address any adverse effects. This may involve pain management using pain relievers, immediate post-procedure fluid resuscitation with saline when necessary, or, if humane endpoints are approaching, a humane termination of the experiment.

A small percentage of animals (5-10%) may die during surgical procedures due to complications related to the procedure or the anaesthesia. Additionally, approximately 20-30% of animals may die due to life-threatening arrhythmias (abnormal heart rhythms) or cardiac rupture (tear in heart tissues) resembling sudden cardiac death in patients with heart conditions. These events are unpredictable and cannot be prevented in advance.

2 Mice will have minor surgery to implant a device under the skin that can release a medicine slowly. They are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in a hospital.

3 Imaging will be performed on animals to monitor the progression of diseases. The procedure is conducted under anaesthesia (unconscious state). The animals are expected to regain full activity within 15 minutes after the procedure.

4 Animals receiving bone marrow transplants may experience a temporary period of illness lasting several days following irradiation. During this time, they may reduce their food intake and potentially

lose weight, up to 10% of their pre-irradiation weight. However, we anticipate that they will begin to regain their body weight about 14 days post-irradiation. The health status of irradiated animals will be closely monitored through daily checks and at least twice-weekly weigh-ins.

5 Single housing can cause stress in mice, but we will minimize the duration if possible. Whenever feasible, we will reintroduce animals to group housing with their original cage mates after a period of single housing.

Expected severity categories and the 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 (67.5%), Moderate (25.1%), Severe (7.4%)

Rat: Mild (5%), Moderate (65%), Severe (30%)

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

A retrospective assessment of these predicted harms will be due by 23 February 2030

The PPL holder will be required to disclose:

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

Replacement

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

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

Currently, there are no non-animal alternatives that can be used to accurately predict or mimic the development of heart disease. Current heart cells used in culture are not mature, and they have limitations to be used to explain heart disease development such as heart attack which is a disease condition occurring in human adults. This is why we need to conduct experiments on animals to explore the fundamental mechanisms and identify potential ways to treat them.

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

We've explored the use of stem cell-derived heart muscle cells as a substitute for mature adult heart cells.

We're currently working on developing a method to grow mature heart muscle cells in a lab dish. This could potentially replace a lot of the experiments that currently use mice. Also, by growing these heart cells in certain conditions, like low oxygen, we might be able to create a model of heart damage without needing to do surgeries on animals, which can cause them discomfort.

Why were they not suitable?

Human-inducible pluripotent stem cell (ips)-derived heart muscle cells as a replacement in our study are not suitable. These cells are not mature cells and do not have mature cell skeletal alignments and associated mechanisms present. We're currently improving the method of the mature heart muscle cells in vitro culture.

A retrospective assessment of replacement will be due by 23 February 2030

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 estimated the number of animals majorly based on our previous data and experience, and results from pilot experiments. We also referred to published data from other research groups, and suggestions from our collaborators.

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

We used the free online tool from NC3Rs (Experimental Design Assistant) to design our experiments to ensure we used the minimum number of animals. We followed 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.

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

We'll carry out a pilot experiment with a small number of animals in each disease model. According to the statistical analysis by integrating the acquired information from the pilot data and past studies, we will calculate the power (the number) of animals to be used. Efficient breeding will be achieved by choosing the right mouse strain and providing a clean and controlled housing environment with proper temperature, humidity, and lighting. Male-female pairs will be introduced at the right breeding age. Detailed and accurate records of breeding pairs, births, and litters will be recorded to track the lineage and genetic information. Lighting cycles will be controlled to mimic natural day and night cycles as this can influence breeding behaviour. The pups will be weaned at the appropriate age (usually around 3-4 weeks) to prevent inbreeding and overcrowding. Genotyping (a method to examine the gene traits) will be timely performed to confirm the genetic characteristics of the mice. New breeding pairs from other sources will be periodically introduced to maintain genetic diversity. Imaging techniques (e.g. Echocardiography is a type of ultrasound scan to create pictures of the heart) will be used in our heart failure model to track the heart function non-invasively, so we can get more data from the same animal. At the end of the experiment, we will harvest as many tissues as possible. We will freeze the samples and make them available to other researchers working on similar questions if the spares are available. Freezing embryos and sperms will be used to maintain the genetic modification breeding with the least live mice.

A retrospective assessment of reduction will be due by 23 February 2030

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 goal of the current project license is to understand the mechanism that governs the progression of CVDs, with a particular focus on heart failure. We employ mice and rats as our animal models, as they have demonstrated high suitability for investigating these disease processes.

We'll use the left anterior descending coronary artery ligation to induce ischaemic heart failure (IHF, a type of heart failure type due to lack of blood supply). The left anterior descending coronary artery is the vessel that provides oxygen and nutrients to the heart. When it's tied off, the heart muscle doesn't get enough blood and oxygen, making the heart difficult in pump blood throughout the body. The artery

ligation requires surgical procedures that will be conducted under general anesthesia and pain management for a comfortable recovery. Continuously, we closely monitor these animals for any signs of discomfort or distress, allowing us to halt the experiment if necessary to minimize any potential suffering. This enables us to terminate experiments based on humane endpoints, ultimately minimizing any suffering endured by the animals involved.

In another model, the animal will be induced to heart failure with preserved ejection fraction (HFpEF). Under this condition, a heart becomes stiff and unable to fill enough blood as it should. The HFpEF model is not expected to cause more than mild distress. Currently, there are no other available heart failure models that involve less suffering.

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

Non-mammalian animals are limited in use due to significant differences in their cardiovascular systems compared to humans. Among mammals, mice and rats are the most useful species for gaining relevant and meaningful pathophysiological information. Mice also offer the advantage of genetic manipulations, enabling us to study the underlying mechanisms.

Embryos and very young animals are not suitable for our research because heart attack and heart failure are medical conditions occurring in adults.

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

During surgery, we will use the Rodent Surgical Monitor (equipment monitoring the vital signs, such as body temperature, heart rate, and breathing, in mice and rats) to continuously track the animal's health and ensure the animal's well-being. If any anaesthesia or surgical issues arise that may endanger the animal, we will immediately terminate the experiment and ensure humane euthanasia.

After surgeries, animals will be placed in individual recovery cages with food and water access. Mice will be re-grouped upon full recovery. Animal behaviour will be monitored hourly before they fully recover and at least twice during the first week after surgery to detect signs and distress at an early stage. Scoring sheets will be used to assess the welfare status of animals and establish early humane endpoints wherever possible.

We'll use imaging to keep track of how the animals' diseases are changing, and they'll be given inhalation anesthesia like isoflurane instead of injections like ketamine/xylazine. This should make it easier for them to fall asleep smoothly, adjust their sleepiness quickly, and wake up faster. It's also expected to be safer for their hearts and breathing, and they should handle it better overall.

We acclimate animals to changes in their environment as needed. For instance, when evaluating individual food intake and metabolic processes, animals may require solitary housing in specialized cages for up to 7 days. Single housing can sometimes induce stress, leading to potential aggressive behaviour when re-introduced to their littermates. To mitigate this, we'll follow recommendations as detailed on the NC3Rs website, utilizing pre-exposure to soiled bedding for several days before re-grouping as a refinement measure to reduce aggression. Animals on a tamoxifen diet may experience

weight loss (10-20%) in the first few days. To mitigate potential aversion to eating, sweeteners or other palatable substances will be incorporated when administering tamoxifen to animals via diet.

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

We adhere to best practices to refine our experiments, including guidance from the Experimental Design Assistant (https://eda.nc3rs.org.uk/), internal guidance developed by the Named Veterinary Surgeons for tamoxifen use, recommendations for pilot studies (https://www.nc3rs.org.uk/3rs-resources/conducting-pilot-study) in the NC3Rs resource library, the ARRIVE guidelines, PREPARE guidelines, and LASA Aseptic surgery guidance.

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

We will regularly monitor the NC3Rs website, subscribe to their newsletter, and participate in Regional 3Rs symposia. We've recently joined the NC3Rs cardiovascular network. We'll stay updated on the newest models for cardiovascular research, participate in network meetings, and offer feedback to help support the cardiovascular research community. We also adhere to the ARRIVE guidelines and stay updated on advancements in the 3Rs, such as those found on the Norecopa platform (https://norecopa.no/alternatives/the-three-rs) and the Danish 3R-Centre (https://en.3rcenter.dk/3r/replacement). Our goal is to swiftly integrate these advancements into our practices during the project.

A retrospective assessment of refinement will be due by 23 February 2030

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?