NON-TECHNICAL SUMMARY

Understanding the cell biology behind cardiovascular diseases

Project duration

5 years 0 months

Project purpose

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

Key words

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

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

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

A retrospective assessment of these aims will be due by 9 November 2028

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

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

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

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

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

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

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

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

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

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

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

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

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

Species and numbers of animals expected to be used

- Mice: 16,050

Predicted harms

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

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

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

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

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

Specifically:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Dosing/Sampling routes:

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

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

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

Number of steps animals undergo/Cumulative harm:

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

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

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

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

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

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

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

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

Similarly, to study aneurysm formation (the bulging of weakened arteries) and rupture (the bursting of bulging arteries) mice need to have aneurysms form and rupture. Mice tend to be unaffected by having an aneurysm in a blood vessel, but rupture leads to sudden blood loss and instantaneous death. As rupture is the key event that kills humans, it is important to study why some aneurysm don't rupture, whilst others do.

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

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

Mice:

Mild = 76%

Moderate = 21%

Severe = 3%

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

* Killed
* Used in other projects

A retrospective assessment of these predicted harms will be due by 9 November 2028

The PPL holder will be required to disclose:

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

Replacement

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

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

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

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

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

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

We consider the use of animal experimentation as the final step in the research programme. Thus, when we use animal models this will be because we typically have several years worth of 'test tube' experiments that support the use of animal experiments for disease modelling.

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

Why were they not suitable?

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

A retrospective assessment of replacement will be due by 9 November 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

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

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

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

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

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

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

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

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

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

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

A retrospective assessment of reduction will be due by 9 November 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

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

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

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

We only use mice, which represent the 'lowest' model animal that can be genetically manipulated, for which reagents are available to analyse outputs, and which generates human-like cardiovascular disease in a suitable time frame.

For example, to generate experimental atherosclerosis, mice are fed a high fat diet for ~12w. Thus, we cannot use immature 'pups'.

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

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

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

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

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

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

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

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

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

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

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

As above, we are always actively searching for newer, better models that may come available (e.g. publications, conferences). More formally we follow the ARRIVE guidelines and keep up to date with other developments and advances in resources such as the NC3Rs (https://nc3rs.org.uk/our-portfolio),the North American 3Rs collaborative (https://www.na3rsc.org/the-3rs/), the Norecopa database (https://norecopa.no/), and the Danish 3R-Centre (https://en.3rcenter.dk/), with the aim to implement important developments and alternatives swiftly.

A retrospective assessment of refinement will be due by 9 November 2028

The PPL holder will be required to disclose:

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