Home Office

NON-TECHNICAL SUMMARY

Promoting proliferation in non-regenerative tissues

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

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;

1. Provide new insights into the mechanisms required for regeneration.

2. 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: 7800

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.

a) Typically, juvenile genetically altered mice will be administered with a virus or agent. This will lead to the expression of a gene (e.g. Cyclin T1) 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 (e.g. Myc) 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.

b) 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 (e.g. Cyclin T1) 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. After recovery from surgery, a non-toxic protein activating agent will be administered by injection. This second agent will transiently activate a switchable protein (e.g. Myc) 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.

1) We will use well-validated systems and mouse models in which we have extensive experience.

2) 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.

3) 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

4) 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.

5) 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.

6) Many of our experiments require animals with complex genotypes. We will carefully plan breeding strategies to minimise the number of animals of incorrect genotype.

7) At every stage, we carefully examine and question our need for animals and have consequently adopted whatever strategies possible to reduce their use.

8) 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.

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?