Home Office

NON-TECHNICAL SUMMARY

Controlling Cell Proliferation in Cancer and Regeneration

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Cancer, Regeneration, Myc, Proliferation, 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?

We investigate genes required for regeneration, but that are expressed uncontrollably in cancers. Our overarching purpose is to develop therapies that either promote controlled repair and regeneration or target cancer cells for destruction.

A retrospective assessment of these aims will be due by 8 August 2031

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?

Tissue regeneration (healing following injury) is a complex process that involves several key biological steps. These include detecting injury, creating physical barriers that isolate the damaged area, allowing cells to re-enter the cell cycle, moving cells to required areas, removing debris, changing cells from one type to another, and reconstructing tissue. Proto-oncogenes (normal genes in cells that, when mutated or overexpressed, can become cancer-causing – ‘oncogenes’), when working properly, are important for regulating tissue regeneration. They help the body sense signals that promote cell growth and division. However, if proto-oncogenes are mutated or overactive, they turn into oncogenes. This change can lead to uncontrolled cell growth and cancer. By studying oncogenes, we aim to understand their role in both tissue regeneration and cancer. This knowledge could help us create new treatments to take advantage of the body’s ability to regenerate cells and to fight cancer.

Regeneration: 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. This proposal will pioneer a transformative cardiac regeneration therapy to eventually eradicate heart failure and its complications, thereby providing the means to impact both the life span and health span of millions of people globally.

Cancer: Cancer affects millions of people worldwide; it is estimated that one in six deaths worldwide is due to cancer. Two cancer types, lung and liver, account for more than a quarter of all cancer deaths. The primary reason for poor survival outcomes is ineffective early detection, resulting in tumours that are harder to treat and that often display genetic features that have no known treatments. The enormous clinical burden of cancer in terms of mortality, morbidity and healthcare resources means it is an urgent priority to develop novel therapies that could transform their treatment.

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

Advances in fundamental knowledge

Our research is centred on understanding how certain biological pathways work during the regeneration of tissues like the heart, and how these processes can go awry in diseases like cancer. We use specially designed mouse models that allow us to quickly turn specific genes or proteins on and off. This helps us understand their roles in normal body functions like healing and in the development of tumours.

Through this project, we aim to make important advances in understanding why organs, such as the heart, struggle to heal and regenerate. By studying how adult mammals can repair their heart tissue, we hope to develop new treatments that promote healing.

Additionally, this project will help us learn more about breaking down proteins in a way that could lead to better treatments for lung and liver cancers. We will also explore new ways to deliver biologic materials—like DNA (the code for life), RNA (a copy of DNA) or proteins (the building blocks of cells)—into tumours and cardiac tissues, which could enhance our treatment options.

Identification and verification of potential cardiac-regenerative and anti-tumour therapies

The project aims to discover and confirm new treatments for cancer. We utilise state-of-the-art therapeutic techniques to create special molecules that can help break down harmful proteins and block the growth of cancer cells. These approaches will help us better understand how tumours might shrink and allow us to assess any collateral damage to normal tissues.

Additionally, the project will explore new ways to treat heart failure. We will investigate whether a similar method can be used to help other non-regenerative tissues like the brain.

Production of valuable resources

We will share our findings at scientific conferences and publish them in respected journals, while also engaging the public in our work. Additionally, we will make our raw data accessible to everyone for further use.

To conduct our studies, we have created special mice with altered genes that can be controlled. As we progress with our research, we plan to develop more unique strains of mice, which will also be available to other scientists. These resources will help others explore how certain proteins function in regeneration, normal tissue, and cancer.

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

Cardiovascular disease is the most common cause of death around the world, leading to more than 18 million deaths every year. A significant number of these deaths—about 85%—are due to heart attacks and strokes. During a heart attack, the heart doesn't get enough oxygen, leading to the death of heart muscle cells. Unfortunately, these heart cells in adults don’t have the ability to grow back after injury, which means that when they die, the heart can’t repair itself. This loss of heart cells leads to weaker heart function and can eventually result in heart failure and death.

Currently, if someone has a heart attack, doctors often perform surgeries like artery bypass grafts to restore blood flow and use medications to manage symptoms. However, these treatments can't bring back the dead heart cells. Heart failure affects over 64 million people globally, including more than a million in the UK, causing both significant health issues and high medical costs. For those with severe heart failure, a heart transplant is the only effective solution. But the number of available donor hearts is very limited, with only a few thousand transplants done each year worldwide (around 200 in the UK). This highlights the urgent need for new methods to help regenerate heart tissue.

This proposal aims to create a groundbreaking therapy that could help restore heart function, potentially eliminating heart failure and its related complications, which could greatly improve the quality and length of life for millions.

Cancer affects millions of people worldwide, and it is estimated that one in six deaths worldwide is due to cancer. Two cancer types, lung and liver, account for more than a quarter of all cancer deaths. The primary reason for poor survival outcomes is ineffective early detection, resulting in tumours that are harder to treat and that often display genetic features that have no known treatments. One such feature is the abnormal levels of the proto-oncogene called Myc, which appears in over 70% of tumours. Myc is an important target for developing new cancer drugs. However, after more than 50 years of research, no traditional small-molecule drugs have been developed to target Myc. Although targeting Myc is challenging, its presence in many tumour types shows how valuable an effective treatment could be for many cancer patients. We urgently need to create new treatment options.

One innovative approach to developing new cancer treatments is called Targeted Protein Degradation, or TPD for short. This method focuses on making it easier to target certain cancer proteins that are usually hard to target, like Myc. TPD uses a specific technique known as PROteolysis TArgeting Chimeras, or PROTACs. Essentially, these treatments work with the body’s natural processes to help remove unwanted proteins that contribute to cancer. Scientists can either modify existing medications or create new ones to tackle these tough targets. Recently, advancements in drug discovery, especially with the introduction of mRNA and viral therapies, like the ones used in the covid vaccines, have opened up exciting possibilities for TPD. One particular area gaining attention is biological PROTACs, or bioPROTACs, which represent a cutting-edge way to develop treatments. Biological drugs are drugs derived either from living sources (e.g. cells) or their components (DNA, RNA, proteins). This new field holds great promise in the fight against challenging cancers.

This proposal focuses on developing new therapeutic prototypes for testing in animal cancer models to see if they can effectively stop tumour growth. Importantly, these investigations will lay the groundwork for further engineered biological molecules. Given the significant impact of cancer on lives and healthcare resources, there's a strong need for innovative therapies to improve cancer treatment.

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

We are committed to sharing our research findings and tools with other scientists, and we plan to publish all our results (positive or negative) in well-respected scientific journals. Our work will be valuable not only to us but also to the biotechnology industry and other researchers looking for new ways to heal damaged tissues and treat cancer.

We also aim to keep an open dialogue with patients and the public to hear their thoughts on the new treatments we are developing.

Our goal is to attract talented postdoctoral researchers and graduate students, helping them grow in their scientific careers through the projects we have outlined.

The data we collect will be made available in public databases so that other researchers can use it. We will share our findings at international scientific conferences and seminars. We will also explore the best ways to advance our work into practical applications, protecting our ideas with patents when necessary.

A successful outcome from this research would be a significant advancement in the fields of regenerative medicine and cancer treatment. In the future, this could lead to new, life-saving therapies for millions of patients.

Species and numbers of animals expected to be used

- Mice: 5750
- Rats: 130

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 mouse tissues. These models have enabled us to understand why some non-regenerative cells do not repair organs after damage and why some organs are cancer-prone. The genome for rats has also been published, making molecular biology studies of rodent species highly practical. These rodent models represent the most suitable system in which to perform these studies.

We are particularly interested in studying adult human tumours, especially those that develop in the lungs and liver. To do this, we use special mouse models that grow tumours in these organs, which are very similar to the tumours found in humans. Regular lab-grown cells don't give us a complete picture because they can’t replicate the complex environment and interactions that happen between tumour cells and various non-tumour cells in the surrounding area of the tumour. Our goal is to find new ways to treat these tumours, so it's important for us to understand how tumour cells interact with surrounding stromal (supporting) cells. Since standard cell models fall short in capturing these interactions, we will primarily be using adult mice in our research.

One non-regenerative tissue is the heart. Following an injury, the adult human 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. The induction of a heart attack (myocardial infarction, MI) in rodents (mice and rats) 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 the investigation of mechanisms for heart repair and regeneration. Cell models in the laboratory do not adequately copy the interactions in which cardiac regeneration occurs.

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

Some female mice (genetically altered or not) will be given a drug containing hormones that cause ovulation of an increased number of oocytes (eggs) than is typically observed in a natural ovulation. This is called superovulation, and it will involve two injections, 48 hours apart, either into the body cavity (intraperitoneal) or under the skin (subcutaneous). For the collection of oocytes, the animals will be humanely killed. If embryos are required, females will be mated first and at the desired developmental time point, humanely killed, and tissues collected.

Some female mice (genetically altered or not) will be mated with a sterile male to produce pseudopregnancy (i.e. making the uterus receptive for implantation of embryos) and then undergo either nonsurgical or surgical embryo transfers. During non-surgical embryo transfer, embryos will be inserted directly into the uterus with a special device without any surgical intervention. Non-surgical embryo transfer will be used whenever possible, as it will cause the least harm. In some cases, surgical transfer may be required, if, for example, there is persistent low efficiency of non-surgical transfers or if it is desirable to transfer embryos at very early stages. This will involve a surgical procedure to place the embryos in the uterus. If surgical transfers are required, the animals will have surgery under anaesthetic and be provided with post-operative care, including pain relief, to minimise pain and distress. When the aim is to generate a new mouse colony from frozen sperm or embryos, live offspring will be born following embryo transfer.

Some male mice that have not been genetically altered will undergo surgery for vasectomy. They will have surgery under anaesthetic and be provided with post-operative care, including pain relief, to minimise pain and distress. These males will be used to mate with females to induce pseudopregnancy (i.e. making the uterus receptive for implantation of embryos). These males may be kept alive to mate with other females. At the end of mating, these animals will be humanely killed. Vasectomised males will be singly housed between matings.

Female and male mice will breed together to maintain a colony of mice containing genes with mutations for study. They will undergo no procedures other than mating and observational handling (e.g. checking health and signs of mating). Before weaning, a small ear punch tissue biopsy will be taken to ascertain genetic status. Matings will be ongoing to maintain the colony, and timed to generate embryos at a particular developmental time point. Stud males will be singly housed between timed matings. For ongoing matings, each female will have up to 6 litters and then will be humanely killed.

After the generation of wild-type or genetically modified animals or the acquisition of mice and rats from other sources, rodents 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. Where surgery is necessary, animals will have surgery under anaesthetic and be provided with post-operative care, including pain relief, to minimise pain and distress. A non-toxic protein-activating agent may be administered by injection. This second agent will transiently (for short periods) 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. Alternatively, 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 from a superficial blood vessel, such as the tail vein, may be taken to monitor mice, which may cause mild and transient pain, but this is expected to be momentary. 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. In adulthood, mice will undergo an experimental heart attack by injury of a coronary artery, which requires surgery to access the heart. In some cases, an agent that leads to gene expression or activation will be injected directly into the heart tissue. They will have surgery under anaesthetic and be provided with post-operative care, including pain relief, to minimise pain and distress. Food may be withdrawn for short periods. Blood sampling from a superficial blood vessel, such as the tail vein, may be used to assess biological markers of MI. Some animals will not be recovered from MI surgery. After recovery from surgery, a non-toxic protein-activating agent will be administered by injection. This second agent will transiently activate a switchable protein (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 84 days, by non-invasive monitoring (e.g. ultrasound scan), 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.

c) Typically, genetically altered mice will be administered with cancer cells grown in the laboratory. Cells will be injected into mice via either subcutaneous or tail injection (so that cells enter the bloodstream), leading to the establishment of tumours, which will be monitored visually or by non-invasive imaging throughout (e.g. optical imaging). The cancer cells may be genetically engineered to express genes that can be switched on and off by administration of a gene or protein-activating agent. Alternatively, a cancer therapeutic will be administered by the most appropriate method. We will label

newly made cells by injecting or feeding a specific labelling agent. Mice will be killed humanely at specific time points or when their tumours reach a defined size.

d) Typically, genetically altered mice will be administered with a virus or tumour-causing agent intranasally (into their nose) or by injection (usually intraperitoneal). Administration of such substances induces tumour development in the lungs, which may be monitored by non-invasive imaging (e.g. optical imaging) throughout the experiment. In most cases, a potential drug will be administered or activated following tumour development by administration of a gene or protein-activating agent or the most appropriate method. Blood sampling from a superficial blood vessel, such as the tail vein, may be used to assess biological markers of tumours. We will label newly made cells by injecting or feeding a specific labelling agent. Mice will be killed humanely at specific time points or when their tumours reach a defined size.

e) Typically, genetically altered mice will be administered with a virus or tumour-causing agent by tail vein injection. Administration of such substances induces tumour development in the liver, which may be monitored by non-invasive imaging (e.g. optical imaging) throughout the experiment. In some cases, a potential novel drug is administered or activated following tumour development by administration of a gene or protein-activating agent or the most appropriate method. Blood sampling from a superficial blood vessel, such as the tail vein, may be used to assess biological markers of tumours. We will label newly made cells by injecting or feeding a specific labelling agent. Mice will be killed humanely at specific time points or when their tumours reach a defined size.

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 injection). Any animals that undergo surgery are likely to experience transient post-operative pain and discomfort, but no significant disturbance of an animal’s normal state. In addition, some animals will experience short-term moderate pain, suffering or discomfort from either extensive activation in proliferation in some organs or tumour development (less than 10 %). 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 2 %); death usually occurs suddenly with only transient suffering.

Our experimental models are designed to either induce proliferation in normally non-regenerative tissues such as the heart and brain or drive tumour cell death. We have developed switchable tissue-specific technologies so that protein expression is localised and transient, which 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.

One scientific goal is to test and develop new treatments to replace lost cells in the heart and brain, and therefore rely on being able to model the regeneration of the adult human heart in rodents. 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 MI in rodents is the most commonly used method for studying ischemic heart disease in mammals and represents the current gold standard for the investigation of mechanisms for heart repair and regeneration. Approximately half of rodent 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 the 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.

One scientific goal is to test and develop new treatments for liver and lung cancers, and therefore, we rely on being able to model human cancers in rodents. Our experimental models are designed to induce tumours in the lungs and liver of mice that will cause the least suffering for the shortest period. Nonetheless, the nature of the experiments will cause inevitable adverse effects, and mice may suffer transient weight loss and tumour development (which in the lung may lead to respiratory distress). Extensive monitoring will be used to detect signs of tumours, and animals will be killed humanely if they display signs of ill health, such as a hunched posture and inactivity.

The enormous burden and severity of heart attacks and cancer in patients warrant the use of this category of animal protocols in order to find new treatments.

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 86%

Moderate 10%

Severe 2%

Non-recovery 2%

Rats Mild 10%

Moderate 50%

Severe 5%

Non-recovery 35%

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 8 August 2031

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 is a very complicated organ. Many different things, like cells, tissues, and blood, all work together to make it beat. Scientists cannot copy how the heart works in a lab because it is so complex.

A heart attack, also known as a myocardial infarction, happens when the flow of blood that brings oxygen to a part of your heart muscle suddenly becomes blocked. If the flow of blood is not quickly restored the heart cannot get enough oxygen and the heart muscle will begin to die.

Following the death of the heart muscle many complex changes occur across extended timeframes:

• Inflammation: The body sends in "cleanup cells" to remove the dead tissue.

• Building Glue: The body creates an extracellular matrix (a kind of biological glue) to hold everything together.

• Scarring: This is called fibrosis, where the heart builds a permanent scar to patch the damage.

Scientists can grow cells in plastic dishes (called cell culture), but these cells do not act the same way they do inside a body, and they do not have the same "team" of cells around them. Therefore, right now, there is no computer or cell culture model that can replace the need for these animal studies to find new cures. Because of this, we use rodents to study heart attacks. We have well-established methods to cause an experimental heart attack, and the hearts respond to damage in a way that is very similar to human hearts.

Therefore, experiments on animals are crucial for learning how the heart functions, how it responds to damage, and for testing new treatments. Currently, there is not a substitute that can completely replace the need for these animal studies.

The same applies to cancer. Cancers are complicated, and the way cancer cells interact with their surrounding environment is crucial for their growth and survival. Studying cancer cells alone in lab dishes does not provide a complete picture of how they develop and respond to treatments.

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

Our animal studies are complemented by cell culture experiments.

We use commercially available and mouse-derived cells grown in lab dishes. We also use three-dimensional culture methods (organoid models) where possible. The muscle cells of the heart, the cardiomyocytes, can be generated from rodent or human embryonic stem cells (cells at very early stages of embryo development), which can be differentiated into specialised cells. These newly differentiated cardiomyocytes model immature (neonatal) cardiomyocytes and so can only be used together with animal models. Adult rodent cardiomyocytes can be purified and survive in culture for a few days.

Our cell culture studies are invaluable for investigating the cell autonomous (where the cell behaviour is determined by its own internal properties) nature of cell signalling, but are unable to address complex interactions between multiple cell types in the surrounding environment.

Why were they not suitable?

Embryonic stem cell-derived cardiomyocytes display very immature characteristics, 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 model the cellular context and interactions in which cardiac regeneration occurs.

Cells in culture are kept in conditions that are very different from those in a living animal. They face changes in oxygen levels, get a lot of glucose, and are often provided with unclear growth factors like vitamins or hormones. While three-dimensional culture methods, like organoid cultures, have some benefits, they are limited by the simplistic and unrepresentative scaffolds (three-dimensional structures designed to support and guide cell growth) used. These methods also cannot recreate the complex interactions between different cell types. Additionally, it is impossible to form a functional immune system that can respond to healing or cancer growth. Therefore, cell culture systems do not effectively model the complex interactions during healing or cancer and are not good substitutes as models.

A retrospective assessment of replacement will be due by 8 August 2031

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?

Estimation of numbers is informed by an analysis of published work and preliminary experiments, and with assistance from a statistician. We will use well-validated mouse models in which we have extensive experience.

In most cases, experimental groups will represent an analysis of gene activation or inactivation over a time course. For example, groups will be at various timepoints after administration (or withdrawal) of a gene-activating agent and/or administration of a specific therapeutic. For surgical animals, controls can include sham surgical animals (animals that have undergone surgery, but no heart attack induced to estimate heart function after no damage), genetic controls and vehicle treatment controls.

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 rodent models in which we have extensive experience. Variability between experimental groups will be limited by using closely related rodent strains raised in a managed environment, free from specific diseases, fed a uniform diet and matched for age and body weight.

2) Our previous studies, and those of our collaborators on mouse models of heart attack and cancer induction, 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 management over variability, reducing the number of animals required per study

4) The experimental group sizes will be based on pre-existing data. Importantly, each animal’s initial measurement can act as its own standard of comparison when analysing non-invasive imaging, ensuring that the experiments do not need to be repeated unnecessarily.

5) Animals 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; medium used to administer experimental agents) and retain a key to identify recipient rodents. This key will only be available after analysis has been carried out.

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

7) At every stage, we carefully examine and question our need for animals and have consequently adopted whatever strategies are 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?

Many genetically altered animals used in this project already exist and will be obtained from the relevant suppliers or collaborators. Genetically altered mouse colonies will be maintained as homozygote whenever possible, to minimise the number of unwanted genotypes. We will keep stocks of frozen sperm and embryos to avoid unnecessary breeding where a line is not required for several months. Since most of our experimental animals have complex genotypes, we have carefully planned breeding strategies to maximise the number of suitable experimental animals and control littermates.

We ensure that our experimental designs incorporate randomisation, blinding, appropriate sample size calculations, and rigorous statistical analysis methods. Advice will be sought from an expert statistician. Pilot studies will be conducted to determine the feasibility and efficacy. All experiments will be documented and reported transparently.

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

A retrospective assessment of reduction will be due by 8 August 2031

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, cancer induction 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. Importantly, by using genetically altered mice in which we can precisely time gene activation or deactivation, we will refine the animal's experience

during experiments, as we are able to precisely interrogate specific genetic pathways in the tissue of interest (heart, lung, liver) without causing disease in other organs.

We have prior experience with most substances we expect to administer as part of these protocols, including optimal dosing strategies and information on tolerability and efficacy. Whenever any new substances are to be used, small-scale pilot experiments adapted from existing data will be performed to confirm efficacy (using assays and humane endpoints appropriate to the substance) and identify any adverse effects to minimise any side effects. Substances will always be administered in a sterile form, where possible, clinical-grade compounds will be used.

Our models generate tumours with predictable latency and growth, thus allowing termination of the experiment at an earlier stage when tumours are still relatively small. The protocols we will use to inject tumour cells are well established and characterised in the field and our lab. Thus, our tumour models mean that most mice are likely to exhibit only moderate adverse effects. For monitoring tumour growth/responsiveness to substances, we will always prioritise non-invasive imaging techniques such as optical imaging, which have minimal adverse effects.

The induction of heart attack (myocardial infarction) in rodents 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. Rodents represent the lowest level of sentience available to study mammalian heart development and disease. Rats allow for two cardiac surgical procedures, which are necessary to assess prototypical regenerative therapeutics at later stages following a heart attack, which are more analogous to the treatment of human patients with heart failure. Surgical techniques and post-op recovery standards will be continuously monitored and revised. Surgical procedures, including preparation, surgery and recovery care, will be performed in keeping with current best practice guidelines (LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery). Peri-operative analgesia will also be used and maintained for as long as necessary in accordance with our experience in using the models.

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

Lower vertebrates like zebrafish and newts can regenerate their hearts, so they are not suitable for studying heart regeneration in adult humans. We need to model adult human heart regeneration using rodents because their physiology is similar enough to humans to provide accurate representations of human diseases. Our understanding of rodent physiology and genetics helps us with this. Cardiac regeneration decreases as mammals develop, so we need to examine adult rodents, not younger ones or animals that are under anaesthesia. Terminally anaesthetised animals are useful to assess short-term responses to tissue damage. However, procedures cannot be solely performed on terminally anaesthetised animals because regeneration and the effect of the therapy need to be monitored for some time after injury.

We also aim to model the development and regression of adult human cancers in mice. Non-mammalian animals are limited in their use because they either do not have the genetic or tissue complexity associated with lung or liver cancer, or because they do not share cell or tissue features of human cancers. Mouse physiology closely resembles that of humans, allowing us to create accurate representations of the disease. This is made possible by our detailed understanding and comparison of mouse physiology and genetics. Tumour formation is a complex process that takes several years in

humans. However, in mice, we can speed up and control this process using various genetic methods, such as turning protein activity on and off. Procedures cannot be solely performed on terminally anaesthetised animals because the progression of the tumour and the effect of the therapy need to be monitored for some time after the start of the experiment.

How will you refine the procedures you're using to minimise the welfare costs (harms) for 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. Once tumours are detected, additional monitoring will be performed.

Staff will be trained and become experienced in surgery. We will carry out surgeries early in the day, allowing intensive monitoring in the afternoon and evening. Animals will be monitored twice daily over the next three days, and every day after that, to ensure we detect any suffering promptly. Animals will be individually monitored using a clinical health score table for distress post-myocardial infarction surgery. When an individual score is reached or if a cumulative score is reached, animals will be killed humanely.

Where tissues are required at very short time points post-myocardial infarction, animals will not be recovered from deep anaesthetised and so experience no discomfort.

Where injections are necessary, we use the appropriate needle gauge sizes and only use needles once.

In the early stages of heart failure, animals are characteristically well and move freely. Deterioration of the heart is accompanied by specific symptoms. Regular observation and post-surgery scoring of the animals allows these symptoms to be used as humane endpoints. As with man, some deaths are sudden and presumed to be arrhythmic (abnormality of the heart's rhythm). 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 events.

Anaesthetics will be used for surgery and for restraint post surgery (to limit pain/harm to the animal when picked up and restrained post cardiac surgery). Since rodents frequently do not show signs of pain, we will administer pain relief medication and, where possible, analgesic 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.

When tracking and analysing how a new therapy moves and distributes inside mice, myocardial infarction or cancer induction does not always need to be induced, thereby reducing possible suffering.

The timing of tumour formation in our models is highly reproducible, providing a robust scientific model essentially free from random accumulation of mutations and allows a reduction in the number of mice required for statistically significant results. Thus, these models result in the least harm to the animals.

Mice with tumours will be regularly monitored and tumours allowed to grow to the smallest possible size to minimise the discomfort of the animal, whilst still being able to collect pre-clinically valuable information to inform future clinical developments.

Specialised bedding will be used when using hairless mice to reduce the discomfort of the animal. Where animals may scratch their skin, we will apply clay, creams or lotions to alleviate discomfort and aid healing.

Flavoured drinks (e.g. Nesquick or sucrose), spreads, or jelly will be used to increase the palatability of substances (pain medication, gene-activating drugs).

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.

We will constantly refine the environmental conditions that our animals live in. Animals are housed according to the best recommendations and enrichment (including nesting material, tunnels, chew blocks and seeds), and where possible, rodents will not be singly housed. We will ensure there is habituation and acclimatisation, use of non-aversive handling (tunnel and cupping), acclimatisation to handling and procedures and optimal handling and interaction with the animals to maximise their welfare. In addition, post-surgery enrichment will be supplied.

When mice are obtained from external sources, a minimum acclimatisation period of a week is used for the mice to settle into their new environment.

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 the 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. We have regular reviews of protocols/experimental design with advice from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer. Additionally, we will receive communication from Named Information Officers.

A retrospective assessment of refinement will be due by 8 August 2031

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?