Home Office

NON-TECHNICAL SUMMARY

Epigenetic reprogramming influences on ageing 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

Key words

Rejuvenation, Cancer, Longevity, Ageing, Epigenetics

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

The primary objective of our project is to understand the mechanisms involved in cellular rejuvenation and assess its potential as a therapeutic strategy.

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?

Age significantly influences the onset of diseases and the healing of complex wounds. Current wound healing strategies, such as antibiotics and dressings, are primarily effective for minor injuries, highlighting the need to explore cellular rejuvenation for larger and more intricate wounds. In blood, the ageing process leads to an increased production of specific cell types, raising the risk of age-related diseases like leukemia and myeloproliferative neoplasms. These chronic conditions severely impact quality of life and lead to complications such as strokes and hemorrhages, with no known cure other than bone marrow transplantation. However, finding suitable donors is challenging, especially for older patients whose blood stem cells are less fit. Cellular rejuvenation presents the possibility of generating healthy bone marrow from affected patients and rejuvenate aged blood cells, potentially improving transplant outcomes.

In the realm of immunotherapy, CAR T-cell therapy involves modifying a patient's T cells to target and attack cancer cells. The success of this treatment largely depends on the quality of the initial T cells; if they are already exhausted, the therapy may be less effective. Therefore, strategies that preserve early-stage cells and prevent exhaustion are crucial. Cellular rejuvenation could revitalize these cells, enhancing their effectiveness and improving therapeutic outcomes.

Beyond blood and immunotherapy, the potential applications of cellular rejuvenation extend to developing therapies that prevent a wide range of age-related diseases, offering a promising avenue for future medical advancements.

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

This project aims to comprehensively characterise the adverse effects of ageing on various tissues, including skin and blood. This characterisation will enable us to evaluate the effectiveness of our cellular rejuvenation strategy through a comparative analysis of aged and rejuvenated tissues. In the long term, our goal is to develop a cellular rejuvenation therapy suitable for clinical application.

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

The skin research will contribute to the advancement of therapies aimed at treating or alleviating symptoms associated with skin cancer, chronic wound healing, hair follicle loss, and loss of pigment in hair.

The blood research will contribute to the development of therapies for treating tumours and blood cancers, such as leukaemia, and improving the current success rate of bone marrow transplants.

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

We have established collaborations with various research groups, each focused on related fields. These collaborations are expected to accelerate our progress and enhance our comprehension of the mechanisms that govern cellular ageing. This, in turn, will contribute to the creation of more potent rejuvenation technologies.

The findings of this research will be shared through publication in peer-reviewed journals or through patenting, especially when commercial potential is identified. In both instances, the resulting papers and patents will be made publicly accessible, fostering interest, attracting funding, and propelling progress in the field of therapeutic rejuvenation.

Species and numbers of animals expected to be used

- Mice: 7000

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.

Mouse models have been extensively employed in immune and blood research, primarily due to their well-established suitability for such studies. The transplantation of blood stem cells is considered the gold standard method for assessing their stem cell function. Additionally, our research group delves into the intricate interactions among blood stem cells, tumours, and their niche, encompassing various cell types and structures such as bones, vessels, and nerves. The complexity of these interactions poses challenges for modelling them in vitro. Moreover, replicating blood flow and the nervous system using cell culture methods proves to be a formidable task.

Mice offer extensive opportunities for genetic manipulation crucial to our work. Access to primary and secondary lymphoid organs, along with peripheral tissues like the lung, is necessary for the proposed analysis, and such access is limited in human studies.

This project aims to identify positive therapeutic effects that rejuvenation may have on skin tissues in wound healing and on blood cells in bone marrow transplants. Juvenile mice will serve as positive controls to assess whether rejuvenation yields the desired positive impact. Aged mice will be

employed, as rejuvenation therapies are anticipated to be most beneficial for human patients aged 60 and older. The relatively short natural lifespan of mice accelerates the study of the ageing process, expediting the development of therapies for human age-related diseases. Significantly, the ease with which mice can be genetically altered allows for the tracking of donor and host cells, facilitating the study of the effectiveness of blood stem cell engraftment — a key indicator for the successful outcome of bone marrow transplants.

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

In skin studies, animals typically undergo a surgical procedure under general anaesthesia, involving a skin punch not exceeding 10mm in diameter. We will evaluate the benefit of rejuvenation on the recovery process. Upon completion of the protocol, mice will be humanely killed and samples collected.

For blood studies, animals will be exposed to irradiation to deplete their current blood system, followed by injection with donor blood cells, commonly administered through a tail vein. Post-transplantation, blood samples will be collected from the animals to assess their recovery and response to rejuvenation. At the end of the protocol, mice will be humanely killed and samples collected.

In studies examining safety with respect to cancer, mice may be injected either subcutaneously (under the skin) or intravenously (tail vein) with cancer cells of interest. Our interests include whether our rejuvenation methods impact the tumour burden and how the CAR T cells react with the tumour cells. Therefore, the animals will be humanely killed while the tumours are still small.

These experiments will help determine the safety and efficacy of therapies which can be progressed to help human patients.

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

In most instances, animals are not expected to exhibit harmful phenotypes. Nonetheless, some animals may have an altered immune system, rendering them more susceptible to infection. Animals with modified immune status will be housed in a barrier environment, thereby minimising the likelihood of compromising their health.

Furthermore, aged mice often display grey fur and increased weight. Additionally, ageing animals may occasionally experience partial or complete blindness, primarily due to age-related vision loss and, less frequently, the development of cataracts or corneal dystrophy. They may also engage in scratching behaviour, resulting in minor superficial wounds. While the loss of vision is not anticipated to affect the mice's behaviour, if it is caused by a cloudy eye, it can lead to discomfort and, in the worst-case scenario, ulceration of the eye.

Animals that have undergone irradiation or treatment with specific drugs, such as tamoxifen, are more susceptible to weight loss. Therefore, these animals will be closely monitored and weighed regularly.

Some animals receiving subcutaneous (under the skin) injection of tumour cells may exhibit discomfort due to the tumour mass, which could impede normal movement.

Animals used in wound healing studies typically experience a rapid recovery from surgery. However, they may display signs of swelling, which typically resolves within 48 hours. The entire wound healing process naturally concludes within 14 days post-surgery without any intervention.

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)?

Model	Severity	Percentage
Mice	Mild	60%
Mice	Moderate	40%
Mice	Severe	0%

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

As we investigate the interplay of bodily systems, it becomes essential to employ whole animals for determining these factors. Mice have long been a staple in laboratory studies, given that the outcomes often prove replicable in larger mammals and, eventually, humans. Attempting to replicate our experiments on small tissue samples is impractical, as it hampers our ability to measure broader microenvironment interactions, particularly those involving the nervous system and blood flow.

Whenever feasible, we resort to alternative methods such as in vitro cell cultures. In this approach, previously preserved cell lines are grown outside a living animal. These cultures prove valuable for cultivating stem cells and simulating the microenvironment in short-term experiments. Nevertheless, the eventual transition to animal research is imperative to comprehensively study the interactive effects within the entire bodily environment.

Moreover, healthcare regulators mandate animal studies to establish the safety and efficacy of therapeutic procedures before their initial application in humans.

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

We have employed various non-animal alternatives to minimise animal usage to the greatest extent possible. Our approach involves utilising advanced 3D cell culture systems for researching mouse oesophageal tissue without causing harm to animals. Additionally, we employ cancerous cell lines in tissue culture to investigate the effects of our methods on cancer. The primary objective is to comprehensively comprehend the fundamental mechanisms of tissue rejuvenation. Animal usage is only contemplated when it is confirmed that such utilisation is essential to sustain valuable research avenues that cannot be pursued through non-animal systems.

Why were they not suitable?

Though our goal is to extract valuable data from non-animal systems, they fall short of replicating the intricate tissue environments like the bone marrow found in real animals. The latter comprise numerous cell types interacting to perform functions like wound healing. Consequently, there exists a limitation in our ability to fully showcase the safety and efficacy of a technique solely through tissue culture systems. To advance therapies to a point where tangible benefits for human patients can be demonstrated more effectively, the inclusion of animals in our research becomes necessary.

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 calculated the required number of animals based on the various experiments necessary to obtain statistically significant results, facilitating the efficient progression of therapies toward human studies. Drawing from our conducted pilot studies, we can strategically design experiments expected to produce valuable data and determine the optimal quantity that can be executed to high standards within a specified timeframe. Furthermore, we minimise the number of animals used by precisely aligning the breeding of mice with the experimental needs, implementing rigorous monitoring of colony size.

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

To minimise the utilisation of animals, our approach involves maximising the use of tissues from each individual animal and storing various samples suitable for diverse assays. Excess tissues will be made available to collaborators and other researchers upon request, contributing to a reduction in the overall number of mice required for research.

The entire program's animal usage will be streamlined through meticulous planning and scheduling of breeding and experiments, ensuring that the minimum necessary number of animals is employed to address research queries and conduct unbiased studies.

Moreover, in previous studies, we have adhered to the NC3Rs guidance and utilised the experimental design tool (https://www.nc3rs.org.uk/experimentaldesign-assistant-eda; https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction). We have also considered the PREPARE guidelines, guiding our animal experiment planning to achieve meaningful results while minimising the animal count. These tools, along with insights gained from prior projects, will be instrumental in our ongoing commitment to reducing animal usage to the greatest extent possible in the proposed project.

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

We will employ standard and efficient breeding techniques to minimise the necessary number of animals bred for the eventual use of mice in our studies. Each protocol and experimental line will undergo pilot studies involving small animal numbers (around 7 to 10 according to statistical power calculations) to ascertain the likelihood of obtaining valuable data in larger studies and to avoid unnecessary animal use. For the majority of experiments, pilot studies have been previously conducted and will not be replicated in this licence; data from these earlier studies will guide the efficient attainment of significant results with the least possible number of animals.

Following the humane killing of animals used in experiments, we will systematically collect and store all available tissues to obviate the need for repeat experiments. Additionally, we may offer some of this tissue to other research groups, promoting widespread discovery and maximising the impact of our research.

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.

Using mice as a model system offers several well-established benefits, including a short breeding time, large litter numbers, a short life span, ease of genetic modification, and straightforward translation to human therapeutics. We specifically employ mice in our research because many desired genetic modifications have already been established in other labs, allowing for immediate comparability with published literature.

When utilising genetically-altered mice, our preference is to employ models that enable us to control the manifestation of disease or harmful phenotypes, thereby reducing the time and severity of such phenotypes. We closely monitor these mice throughout the experiments. The majority of the mice we

use are not anticipated to exhibit adverse effects, and the procedures we perform are designed to be minimally invasive, with an emphasis on avoiding long-lasting harm.

In our wound healing experiments, we employ purpose-built skin punches designed to collect tissue quickly, creating up to two wounds per mouse. These resulting skin wounds are small, promoting rapid healing and minimising animal discomfort. We restrict the size of skin punches to a maximum of 10mm in diameter, and the procedure is exclusively performed under anaesthesia and analgesia to minimise pain for the animals. Additionally, aseptic bandaging may be applied to prevent wound deterioration while allowing the animals to move freely.

For experiments requiring the irradiation of mice to eliminate specific cells and alter blood cell composition, we adopt a strategy to mitigate suffering. This involves splitting the irradiation and co-injecting helper cells.

In the investigation of immune cell and tumour interactions, some mice will be subjected to the development of skin tumours. Close monitoring is implemented to minimise the duration of illness in these animals, and humane euthanasia is employed if the tumours exceed a size of 15mm.

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

Our research necessitates the use of mammalian animals to accurately emulate various aspects of human biology, such as the skin and immune system. Mice offer the advantage of easy genetic modification, and their well-studied disease states enable experiments to generate valuable data more rapidly and efficiently compared to other animal models.

Our experiments necessitate the use of bone marrow stem cell transplantation which involves the animals being irradiated at the start, to deplete their existing blood system. One of our readouts includes engraftment efficiency. Unfortunately such experiments are not possible in less sentient animals such as zebrafish or drosophila.

In humans, the processes of wound healing and immune function undergo significant changes with age. Therefore, it is crucial to investigate therapies across different stages of mammalian lifespans, necessitating the use of live organisms. Given that cellular therapies for diseases require days to weeks to take effect, animals must be kept alive during these procedures.

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

In studies on wound healing, the size of skin punches will be limited to a maximum diameter of 10 mm, with a maximum of two punches per mouse. The procedure will be exclusively conducted under anaesthesia and analgesia to minimise discomfort for the animals. Additionally, aseptic bandaging may be applied to prevent wound deterioration while allowing the animals to move freely. Post-procedure, mice will be monitored for signs of pain, and analgesia will be administered as needed.

To explore the potential of cellular rejuvenation in enhancing the long-term survival and engraftment of blood stem cells, animals will undergo irradiation at the beginning of the protocol to deplete their existing blood system. Subsequently, they will receive injections of donor blood cells. The irradiation

dose will be administered in a split dose to mitigate the potential risk of severe tissue damage from the conditioning regimen.

In ageing studies, endpoints such as behaviour, body weight, appearance, and biochemical markers will be used to assess markers of ageing. This approach ensures that animals are not subjected to prolonged pain until death from old age. These markers will be continuously monitored to ensure the well-being of mice, and humane culling will be implemented to prevent unnecessary suffering if necessary.

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

We will adhere to guidelines pertaining to record-keeping, surgery, education, training, and reporting of experimental results. Additionally, we commit to following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, which encompass aspects such as study design, randomization, bias prevention, and statistical analysis of results.

For all procedures and animal care, we will strictly adhere to the guidance notes and webinars provided by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) (https://www.nc3rs.org.uk/welfare-assessment) and those from the Jackson Laboratories (https://www.jax.org/news-and-insights/jaxblog/2016/march/experimental-design-top-four-strategies-for-reproducible-mouse-research).

In the context of cancer studies, we will refer to the guidelines outlined by Workman et al. in "Guidelines for the welfare and use of animals in cancer research" (2010, Br J Cancer 102(11): 1555-1577. doi: 10.1038/sj.bjc.6605642).

For management of aged mice, we will refer to guidelines outlined by Wilkinson et al. in “Progressing the care, husbandry and management of ageing mice used in scientific studies” (2020, Sage Journals 54 (3): 225-238. doi: 10.1177/0023677219865291).

By consistently following and consulting these guidelines, we aim to contribute to a culture of transparent, efficient, useful, and reproducible research. This commitment is essential in minimising animal suffering and ensuring that any such suffering is only justified in instances where it is necessary for significant contributions to medicine and science.

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

We will consistently check the NC3Rs website to stay updated on the latest information and advice regarding the refinement of our procedures and the minimization of suffering. Additionally, we will refer to the RSPCA website (https://science.rspcs.org.uk/sciencegroup/researchanimals) for additional information. Our mice will be managed by highly experienced technicians in our Institute who employ the tunnel handling method. These experts in animal experimentation will be consulted regularly to ensure the strictest standards in mouse handling are consistently followed.