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Home Office

NON-TECHNICAL SUMMARY

The role of redox signalling in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Protein tyrosine phosphatase Signalling, Cancer, Redox biology, Biosensor, Immunology

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 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?

Our aim is to increase our understanding of the molecular signals that allow the cells in our bodies to talk to each other and work together, that when deregulated can cause or worsen diseases such as cancer, or promote damage associated with limiting healthspan.

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?

A better understanding the biological mechanisms underpinning human health and the causes of disease will allow us to improve the way we treat disease in the future. It will reveal new strategies or therapies with as few undesirable side-effects as possible.

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

The expected benefit from this project is that we will gain a better understanding of the complex molecular and cellular mechanisms that underpin a healthy lifespan that - when deregulated - can cause or exacerbate complex diseases such as immuno-deficiencies and cancer, as well as age-related decline. Our findings will be published in open-access journals. In the long term, the knowledge generated from this project might contribute to the development of more effective drugs.

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

The immediate beneficiaries are the scientific community and the interested general public who will gain new understanding from our open access publications, presentations and conferences and public engagement activities. In the longer term, the pharmaceutical industry may benefit by exploiting the knowledge generated to develop new therapies. Eventually, society as a whole may benefit from the improved health brought by such interventions.

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

We will publish our findings in open-access journals accessible to all and disseminate our results at international scientific conferences. We will collaborate extensively with other laboratories around the world to maximise the impact of our work. We will make our research accessible to the general public, with the help of our public engagement team.

Species and numbers of animals expected to be used

- Mice: 2000

Predicted harms

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

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

We will use mice, as they are the best understood and most widely used mammalian laboratory animal, with excellent means for generating and interpreting the effects of genetic modifications. Most of the mice will be used at young adult life stage, to study the development of the immune system or tumour development.

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

The majority of mice will be used for the generation and maintenance of genetically modified strains or for the collection of cells and tissues for analysis after the animals are humanely killed. Some mice will be injected with agents to induce superovulation. Some mice will be given single injections of tumour cells resulting in tumours under their skin. To modify gene expression some animals will be administered gene inducing agents such as doxycycline, either by injection or in their drinking water. The tumours will be monitored for size and mice humanely killed once the tumours reach a pre-determined size. Some animals with tumours may be given pharmacological or therapeutic agents to affect tumour growth. Tumour studies typically last 2-5 weeks.

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

The main adverse effect could be a mild and transient discomfort, for example following injection of tumour cells or following surgery to transfer embryos. Tumour bearing mice should not experience clinical signs other than tumour growth under the skin. Should a palpable tumour not grow within 4 weeks, mice will be killed and excluded from the study. Repeated dosing, orally or by injection, might rarley lead to stress (due to increased handling) or irritation at the injection site. This might be expected to impact <10 mice overall. We will prioritise the least invasive methods. Adverse effects, such as pain, can affect animals undergoing surgery (e.g. embryo transfer/vasectomy), however, analgesics will be administered in consultation with the facility vet. Injections for superovulation could cause transient distress but should not cause lasting harm. All animals will be killed by a quick and humane method at the end of experiments.

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 vast majority of mice will experience sub-threshold or mild severities at worst from breeding procedures and will be maintained in our excellent animal facility. Mice are not expected to show any overt clinical signs due to their genetic modifications. The surgical transfer of embryos to surrogate mice (used to generate new genetic strains) may cause short-lived post-operative pain and discomfort categorised as moderate severity. However, non-surgical transfer methods will be used wherever possible. Tumour-bearing mice may experience moderate severity (<5%), if subjected to repeated (e.g. >1 week) handling and dosing of pharmacological agents (e.g. twice daily).

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

- Killed
- Used in other projects

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?

Physiological context is important in our research, because we study biological processes that affect tissues and the immune system, as well as cancer. The complexity of these processes cannot be adequately modelled by other means such as tissue culture, purified proteins or computational methods. Thus, we need to use animals, and in order for our data to be relevant to human biology, we need to use mammals rather than non-mammalian species in which these processes are very different. The mouse is the mammalian species most widely used, most amenable to genetic modification and best understood for such research. Thus, we must use tissues or primary cells isolated from mice and research involving experimentation in live mice, such as tumour growth, to achieve the aims of our project.

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

We use cell lines (2D and 3D cultures)ultures), organoids, and purified proteins widely and wherever possible to study the protein tyrosine phosphatase signalling network and redox biology.

Why were they not suitable?

Tissue culture and purified proteins are very valuable in some aspects of our research. However, there are limits, as some cell types, e.g. from the immune system including T cells, cannot be cultured for long periods, and their protein composition cannot be modified other than by genetic means. Furthermore, tissues and tissue culture experience very different environments, therefore monitoring changes in the cell environment will be more physiologically relevant in tissues or primary cells. To understand this context, experiments on live mice, or using cells and tissues from mice, are the only options. Furthermore, we have found significant differences in the signalling in the same cells in tissue culture compared to grown as 3D tumours. The 3D environment much more closely resembles patient tumours.

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 consulted with our Biostatistician, and have built on our previous experience of working with mice to ensure we use the optimal number of animals in order to make reliable scientific conclusions and avoid the unnecessary use of animals beyond those needed. We estimate that for each breeding pair, we will generate an average of 4 offspring (half of females will fall pregnant with an average litter of 8), and use mendelian frequencies of genotypes to calculate the number of pairs to set up for a particular experiment or colony maintenance. Furthermore, we have used pilot studies to help determine the size of experimental groups for tumour studies.

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

We have used the NC3R's Experimental Design Assistant to ensure we are considering all relevant aspects of design.

Our work is complemented by tissue culture systems, which can involve using cells from a mouse and growing more cells for our analysis, or using cell lines that are already established. We are also exploiting technical improvements to characterise the immune cells of our animals. This means we can obtain more information from a single animal, reducing the overall number of animals required. For our work with the redox biosensor strain, we plan to harvest multiple tissues/cell types using a specialised preservation technique. This means we will increase the amount of data we can derive from each animal, further reducing the overall number of animals required.

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 continue to use efficient breeding to ensure we do not over-produce mice. In addition to pilot studies and harvesting multiple tissues from mice.

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.

Other than for tumour studies, mice will be used for isolation of primary cells and tissues after they have been humanely killed, minimising the pain and distress of animals. The minimum number of

animals will always be used that yield meaningful results, and with the lowest possible relevant severity procedure to address a specific question.

We will work in cell lines to evaluate some effects of genetic modifications prior to generating new mouse strains. In new genetically modified mouse strains, we will undertake pilot studies and test isolated primary cells to investigate effects prior to using experimental models that rely on live animals.

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

The mouse is the best model organism to address our aim and objectives, as its physiology and disease processes are sufficiently similar to humans to allow us to draw meaningful conclusions, and because a wide knowledge base and many genetically modified strains and protocols exist that allow comparisons of results between projects and research groups.

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

Our routine refinement methods include added enrichment for breeders, alternative bedding for animals with reduced motility, using established social groups where possible, habituation to handling, and providing food in gel format, additional warmth and more frequent monitoring for mice at increased risk. For established strains, we only take samples from mice from the first litter for genotyping, and we use ear rather than tail samples. We may introduce further refinement methods to protocols or husbandry methods in consultation with animal technicians and veterinary staff.

Stress and suffering of mice undergoing procedures will be minimised by observation and adherence to clear guidelines on clinical signs that trigger the end of an experiment. In rare cases where it will be required that we induce and maintain general anaesthesia, we will use modern anaesthetics and continuous monitoring.

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

We will follow PREPARE and internal guidelines.

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

Through our AWERB liaisons, who keep us informed of NC3Rs seminars and events.