Home Office

NON-TECHNICAL SUMMARY

The phosphoinositide network in health and disease

Project duration

3 years 0 months

Project purpose

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

Key words

Phosphoinositides, Cancer, Metabolism, Ageing

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is 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?

We aim to better understand how a wide-spread cellular signalling network that works inside cells, called the phosphoinositide network, functions and controls diverse and essential processes like metabolism, the rate of ageing and tumour progression.

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?

The impacts of ageing are both a direct loss of overall robustness with age and also a big increase in susceptibility to a variety of diseases that are known to be more frequent in the elderly, such as cancer, metabolic disease, arthritis and dementia. The increasing proportion of global and UK populations that are elderly is a major social challenge. Our work aims to find some of the molecular reasons why and how there is loss of function and increased susceptibility to disease in the elderly and if there might be connected explanations for the emergence of cancer and metabolic disease in some people. This body of work has the potential to define precise targets for new medicines to slow or reverse these processes.

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

Publications, patents, new knowledge and the early stages of developing new clinical approaches to slow or reverse metabolic disease, tumour progression and ageing.

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

The UK will benefit from the work and the potential biomedical investment flowing from our outputs. The scientific community and commercial sector will benefit from increased knowledge and understanding in the short to medium term. In the longer-term patients will see improved health outcomes resulting from better strategies to treat age-related metabolic diseases such as diabetes and also more selective drugs, with fewer side-effects, to treat cancer generally and prostate tumours particularly.

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

We collaborate with, and disseminate knowledge to, academic and commercial colleagues. We publish our findings when they are both positive and negative. We engage with the public through a variety of

means including presentations or installations at Science Festivals. We host local and international students and schools' projects to explain our work.

Species and numbers of animals expected to be used

- Mice: 11,980

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.

Our work will exclusively use mice. They are a very well validated model for many mammalian processes that are important in humans including ageing, metabolism and cancer progression. Our planned use of mice will enable us to use directed specific genetic modifications and potential medicines to determine how the phosphoinositide network operates and controls ageing, cancer and metabolism and whether we can modulate the network to improve health. These things cannot be done with humans or human cell lines because of ethical considerations or because of the fact cell lines cannot recapitulate many aspects of cancer progression, metabolic disease and ageing that are seen in whole mammals. Other than creating and maintaining genetically modified mice our work will only use adult and elderly mice.

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

The large majority of animals used are used for breeding and maintenance of genetically modified strains that have no or very mild phenotypes when they are looked after inside a carefully run specialised animal-facility.

- A number of mice will require ear biopsies for genotyping.
- A very small number of mice will require recovery surgery.
- A small number of mice will be aged, up to a maximum of 24 months, and will be watched especially carefully to ensure that no unexpected health problems emerge and will be humanely culled.
- A small number of aged or young mice will be fed modified diets, for up to a maximum of 20 weeks, that will lead the mice to become over-weight and experience metabolic disease a bit like diabetes, they will be closely observed so that no unexpected or significant health problems emerge and will be humanely culled.
- A small number of young and aged mice will receive a small dose of sugar by injection into the peritoneum (belly) to test their metabolic health.

- A small number of aged and young mice will receive an injection (either under the skin on the flank or into the peritoneum) of insulin to test their metabolic responsiveness
- A small number of mice will be bred to have a combination of genes that will lead to the slow development of prostate cancer and will be observed very carefully to ensure that the mouse does not experience significant symptoms, will not be allowed to progress beyond 16 months, and will be humanely culled.
- A small number of mice will be injected with tumour cells under the skin of the flank. The mice develop small tumours relatively rapidly but will be culled as soon as a reliable measurement of the rate of tumour progression can be made, much previous work shows this varies between 2 and 3 weeks depending on the tumour cell line. The well-being of the large majority of these mice is not impacted by the tumour.
- Potential medicines we use to treat a small number of mice, or further genetic modifications we introduce into mice, we expect to reduce or stop the ageing process, the emergence of metabolic disease or prostate cancer development.
- No mice will be reused in experiments.

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

For most of the mice, including immunodeficient strains (that is mice with a specifically weakened immune system), we do not expect any impacts or adverse effects in our high-quality specific pathogen-free (SPF, that is environment without any disease-causing microbes) animal care facility. NOD SCID Gamma (NSG) immunodeficient mice can exhibit progressive hearing loss that can be profound at three months of age.

Embryo transfer and vasectomy are surgical procedures with short term post-surgical pain. Post-surgical pain will be controlled by giving pain relief and any animal not fully recovered (eating, drinking, return to normal behaviour) within 24 hours will be euthanised.

Although ageing is a major risk factor for adverse effects, we know that the vast majority of our aged mice remain healthy throughout the duration of their lifetime. There is an increased incidence of adverse effects not observed in young wild-type mice including diarrhoea, eye abnormalities, abdominal swelling movement issues, tremors and seizures. A tiny minority of these develop tumours, but regular checking by our experienced animal technicians ensures these are detected early, and the mouse euthanised immediately. A specific code of practice for caring for aged mice is in place.

Tumour progression following sub-cutaneous (under the skin) inoculation with tumour cell lines will lead to emergence of a tumour with a humane end point being reached within a maximum of 3 weeks. For some cell lines it will be quicker. Progression is consistent (does not vary significantly between mice and normally has no impact on the well-being of the animal.

Tumour progression in models of endogenous mouse prostate tumour progression following conditional loss of PTEN (a tumour suppressor gene, the loss of which causes helps cancers grow) is very slow and mice do not experience any impact on their wellbeing as a result of the genetic alteration for the first 6 months. The majority of our mice will be culled before they reach 6 months. A small

proportion will be allowed to progress to a maximum of 14 months. In this model there is no evidence that the tumour spreads to other tissues and the large majority of animals will reach 14 months without exceeding mild signs. About 2% of mice may display moderate symptoms.

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)?

Subthreshold 75.1% (9000). Mild 23.1% (2764). Moderate 1.8% (216).

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?

Studying how the phosphoinositide network (a ubiquitous intracellular signalling system) regulates the rate of ageing, metabolism and tumour progression, with the aim of improving patient outcomes in these areas, must entail use of animal models of these processes as they are all based on the organismal level cooperation between cells and tissues in a way that cannot be replicated in vitro or in silico. Where possible we have and will use cell culture and organoid systems to substitute for experiments with animals. Systems biology approaches based on computer modelling of signalling, including the phosphoinositide network, are now being applied and our lab has used these techniques to make important advances, however, none of these models is capable of dealing with the many interactions between tissues and different cell types that occur during ageing and tumour progression for example.

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

We are using mouse-derived prostate organoids, human prostate-derived cancer cell lines and many other cultured cell lines as well as computational approaches to better understand how the phosphoinositide network operates at a cellular level. The results from these past and on-going experiments contribute to the evolution of our hypotheses and models that aim to understand how the phosphoinositide network functions in vivo. However, our results show that the phosphoinositide network does not function the same in mouse prostate organoids as it does in vivo and further that it does not function the same in cultured cells as it does in organoids.

Why were they not suitable?

Our published results show that the phosphoinositide network does not function the same in mouse prostate organoids as it does in vivo and further that it does not function the same in cultured cells as it does in organoids.

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?

The numbers of mice required for the generation of modified mouse strains are based on the standard operating procedures extensive experience and literature review. The numbers of mice required for the breeding and maintenance protocols is based on estimations of mouse strain numbers, experience at sustainable colony management practice and the frequency of required genotype combinations. The numbers of mice required for individual experiments are based on power calculations and statistical modelling. We input the known statistical properties (phenotype average and variation), decide upon the minimal effect size acceptable from the experiment from a biological perspective and hence calculate the appropriate group size. The number of experiments required within each protocol is based on the assumption of all go-no go decisions being positive and successful grant funding achieved.

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

We use the NC3Rs Experimental Design Assistant where possible and take advice from the our organisation's statistician about the most effective ways to achieve statistical power without increasing the number of animals used. We work to minimise variation where and when possible. This includes ensuring related experiments are conducted at the same time in the day every time, so that we ensure mice are at the same point in any night/day cycles in their behaviour and metabolism.

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

We use efficient breeding programmes that are fitted to the numbers and frequency with which mice are needed for experiments. We take multiple samples from mice to ensure we can repeat assays if needed, share their tissues and be used for other assays to be performed. We use pilot studies to ensure that our analyses sample biological effects in the optimal context (e.g., dose or time of exposure).

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.

Mouse models are advantageous for biological discovery. They are small and easy to breed, reaching sexual maturity within two months from birth, and have the capacity to produce large numbers of embryos. Furthermore, mice are the best animals for our research because they can provide all the genetic modifications we require. Our gene alterations aim to change the way the phosphoinositide network operates to allow us to understand its role in ageing, metabolism and tumour progression and we do not anticipate they will produce any unexpected deleterious outcomes for the mice.

Most animals on this project are not expected to be subject to any pain, suffering, distress or lasting harm.

Some of the animals (<5 %) in the project will be maintained for a long period of time to investigate ageing. There are no specific impacts or adverse effects expected on mice ageing healthily during this project, and regular monitoring of aged animals will prevent for any unnecessary animal distress.

To study metabolism we will use extremely well validated and understood methods.

The majority of our studies of tumour progression will end before the mice experience any observable signs of disease. The small proportion that allow further tumour progression will be closely monitored and experiments will end before disease progression reaches levels set by international guidelines for studying cancer in mice.

Animals will be habituated to being handled and weighed regularly during protocols to study acute metabolic challenge (6) and tumour progression (7 and 8).

For novel substances and/or routes, a pilot study of a small number of animals would be performed with input from the NACWO (Named Animal Care and Welfare Officer) and NVS (Named Veterinary Surgeon).

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

The choice of species is limited by the fact that invertebrate species do not have a phosphoinositide network that is comparable to that in humans nor do they age, regulate their metabolism in the same way and experience related tumour progression processes.

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

We will follow the latest advice for specific procedures, and we will bring in external experts when needed to refine our methods. We will listen to feedback from experienced PIL (Personal Licence)

holders running the experiments, particularly with regard to monitoring and welfare.

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

All experiments which will integrate refinements from the NC3Rs (e.g., the ARRIVE guidelines and aim to work to the PREPARE guidelines (norecopa), the LASA aseptic guidelines, LASA Diehl guidelines on volumes and frequency limits (Diehl et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. 2001 J. Appl. Toxicol. 21, 15-23) and the most up-to-date veterinary knowledge. We work to the HO guidelines for efficient breeding. In our work with mouse models of cancer we will follow guidance on end points as described in Workman, P., Aboagye, E., Balkwill, F. et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer 102, 1555–1577 (2010). https://doi.org/10.1038/sj.bjc.6605642.

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

We will actively stay updated with our field of research through collaboration, conference attendance and reading the literature. We will take particular note of any technical advances that enable reduction, refinement or replacement in our experimental design. The local mouse facility is also a key source of knowledge, transmitting the latest information on the 3Rs to researchers. Internal protocols are shared across the organisation, enabling rapid uptake of any improvements to the method across groups.

Our NIO (Named Information Officer) is pro-active in sharing 3Rs updates on a monthly basis through a newsletter.