Home Office

NON-TECHNICAL SUMMARY

Investigating the pathobiology and therapeutic opportunities of ubiquitin ligases

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

cancer, Parkinson's disease, mitochondria, protein homeostasis, therapeutics

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

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 aim of this project is to discover the roles played by proteins, known as ubiquitin ligases, in different cell types and how their malfunctioning causes diseases and test whether they can be used to cure or treat disease.

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?

Ubiquitin ligase are cellular proteins of fundamental importance in the cells of our body. They are enzymes that modify other proteins and in doing so, they regulate many different processes that control the health and proper functioning of cells. The poor functioning of these enzymes is at the root of many different diseases, ranging from cancer to neurological diseases to infertility. By understanding the functions of ubiquitin ligases, we can discover a way to prevent or treat these very different diseases. Ubiquitin ligases are remarkably specific and influential in their actions within the cell. We will investigate whether they can be used in disease settings to help reduce or cure disease.

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

The outputs we expect to create include new knowledge in understanding how a class of proteins, known as ubiquitin ligases, affect the ability of cells to control their metabolism and the production of their proteins. We expect also to see how defects happening in a small population of cells affect the overall health of the animal. We will also discover how new chemical and biological treatments designed based on the biology of ubiquitin ligase can change how a disease happens in the body. We anticipate this will lead to publications in scientific journals as well as potentially to new products that could lead to new treatments for patients. We could provide data to support the design of future clinical trials for patients with parkinsonism or with Parkinson's disease.

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

The main beneficiaries of these outputs will be the scientific research community who work on disease mechanisms of different diseases, including cancer and Parkinson's disease. In the short term, publications will inform the direction of other research projects and drug discovery approaches centred around ubiquitin ligase biology. Our work will also aid the clinicians who treat patients with a family history of Parkinson's disease. In the long term, we aim to use our knowledge to design therapies arising from ubiquitin ligase biology.

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

We make our results available in a timely fashion in Open Access publications, and our research datasets are deposited in publicly available and searchable databases, linked to our laboratories website and our institutes' data repositories. We will also publish our preprints on biorxiv, which makes our work publicly available even before peer review. Our results will include negative and unexpected experimental findings. We have also presented our work at national and international conferences and use these opportunities to collaborate and share results and ideas with other researchers in similar or overlapping research fields.

Species and numbers of animals expected to be used

- Mice: 2500

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 are using mice to study what happens in specific tissue types when they lose expression of a ubiquitin ligase. It is not possible to study this effect in a simpler system because the cellular processes we are studying are complex. These include the production of sperm in the testes and the effects of nerve cells dying on a mouse's movement and coordination. There are no cell culture systems that replicate the sperm-producing capacity of the testes. Starting in adolescence, people with mutations in their *FBXO7* gene develop a progressive parkinsonism which affects their coordination along with other symptoms. The nerve cells of mice that do not have Fbxo7 will eventually die, and the mice will experience movement deficits slowly between 5-30 weeks of age. Therefore, these mice can be used to study some features of the human condition. Moreover, these mice are unique, as these clinical signs are not observed in less sentient animals.

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

Mice will be specially bred to bring together particular combinations of genetic mutations in ubiquitin ligases which may lead them to develop diseases, like male sterility or nerve cell death. Mice may be injected in a surface vein with viruses containing a specific gene to test for the ability of that gene to slow or stop the death of nerve cells. Neither the injections nor the viruses cause diseases but are instead aimed at treating the diseases that result from the mutations in ubiquitin ligases. These diseases typically take between 5-30 weeks to manifest, and some mice will be injected during this time frame. Animals will usually undergo one procedure, usually being genetically modified, and approximately 75 mice will also be injected with viruses, undergoing two procedures.

Mice may have tumour cells injected into them under their skin to allow a tumour to grow. These tumours may be treated with new therapies delivered by injection, either in a surface vein or into the tumour, to slow or stop the tumours from growing. These experiments normally take between 4-12 weeks, with tumours taking about 2-8 weeks to develop, and injections taking place over 1-2 weeks

after tumours are established. A maximum of 100 mice will undergo one procedure of being injected with tumour cells and allowing tumours to grow. Of these animals, about 50 may be injected with a therapeutic based around ubiquitin ligase biology to test the ability of slowing or stopping tumour growth. Others will be controls.

Mice will be humanely killed using several methods. Different methods are used so we obtain brain tissue ideally suited to use in laboratory tests (*in vitro*) once the animal is dead.

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

The majority of the animals on this licence will experience no adverse or mild effects, like male infertility and anaemia. Some mice with loss of Fbxo7 limited to neurons will develop mild defects with motor coordination. A few mice will develop tumours, as we test novel agents for their ability to stop or reduce tumour growth.

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 38%

Moderate 62%

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

- Killed
- Used in other projects
- Kept alive

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?

It is possible to generate some types of information about how some aspects of organ (e.g., brain) and tissues develop using cell cultures. However, it is not possible to fully mimic the normal and disease process in the laboratory (*in vitro)* as these experimental systems do not fully replicate the complexity of cell interactions or disease processes seen in an organ or a whole animal (*in vivo)*. At present, we cannot use cultured cell systems to ask how a particular protein functions in complex organs such as the testes or brain, where multiple different types of cells exist and interact to enable the organ to

function. Mice have similar developmental processes to humans, so to understand the normal and disease processes in complex tissues, it is appropriate to use mice where they can effectively model aspects of human diseases. Similarly, it is not possible to study tumour biology, and the complex contributions of the tissues surrounding a tumour to its survival in cell culture systems. For this reason, a tumour model is necessary. Also, to develop therapeutic approaches with the potential to treat human diseases it is necessary to establish their efficacy and toxicity in our animals. We will remain alert to any advances that will enable the replacement of animals in our work.

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

We will, where possible, gather as much data as possible using *in vitro* assay systems. This may include more tractable cell culture systems, for example, that model organs by allowing growth in three dimensions or stem cells differentiated to neurons. We will also use patient skin cells to study aspects of cell biology and, where possible, reprogramme these to nerve cells, to address their specific functions, like growing specialised nerve structures, called axons. In parallel, we will utilise gene-editing technologies to introduce mutations into human and mouse stem cells in culture to make greater numbers of nerve cells, which reduce the need to generate new lines of genetically altered mice.

Why were they not suitable?

The induced neuronal cell models are not very efficient, and do not always differentiate to the specific sub-type of neuron that dies in Parkinson's disease and which we aim to study. Also, none of the *in vitro* models reflect whole animal physiology or neurodegenerative processes, e.g., like the impaired movement or the loss of connections between different regions of the brain which we see in the mice. There are no cell models that recreate the processes required to create the final shape and functionality of sperm cells. It is not possible to study these dynamic processes in cultured cell lines.

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?

Our use of mice to investigate aspects of Parkinson’s disease require the use of mice bred with genetic changes. A number of genes must be inherited simultaneously in a mouse for the expression of Fbxo7 to be lost in neurons. Based on this, approximately 1 in 8 pups born will lack Fbxo7 expression in neurons. We estimate our numbers based on 5-6 breeding pairs having 5-6 litters of approximately 5-6 pups to generate mice for experiments. For mice experiencing infertility due to loss of Fbxo7 expression, 1 in 4 pups born to a breeding pair will lack Fbxo7 expression and only half will be male, so 3 breeding pairs is sufficient to generate mice for these experiments.

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

We have studied the Fbxo7-deficient mice for 5 years and found the clinical signs to appear in 100% of the mice that have mutated Fbxo7 genes, which reduces the number of mice needed for studies to generate statistically significant data.

We will perform pilot studies testing any new therapies in very small groups of normal mice. These new therapies will have already been tested in isolated cells in the laboratory. These small studies will allow us to determine the effectiveness of new therapies on tumours and minimise the suffering of these animals. We consult the advice offered in the PREPARE guidelines (https://norecopa.no/PREPARE) for planning our experiments.

We also use resources for study design on the NC3Rs website, which includes the experimental design assistant, and advice on conducting pilot studies, reporting, and sample size calculations.

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

Efficient breeding is the main measure that we use to reduce the number of animals used. Breeding pairs will be replaced after 6 litters and those with small litter sizes or husbandry issues will be replaced. Where possible, we will share tissue and mice with other labs that want any material from us, as we have done in the past.

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.

We plan to test the functions of ubiquitin ligases with clinical relevance (i.e., where analysis will directly impact our understanding of human diseases). We will use two different genetically altered strains of mice for these studies, one that results in reduced Fbxo7 expression in all cells of the mouse and the second limits this loss only to specific neurons. In both strains, most mice do not experience any adverse effects and a small number of mice will develop mild or moderate clinical signs as they age.

We also plan to test the effectiveness of new therapies based around ubiquitin ligase biology in tumour transplantation models. The mice used are well-established and allow for the measurement of multiple characteristics of tumour biology, including the capacity of the cancer to spread throughout the body and the growth of new blood vessels within the tumour.

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

We need to use adult mice since the neurological signs develop over time, starting at 5 weeks and continuing to 30 weeks of age. Additionally, male mice only become sexually mature and start to produce sperm at 10 weeks of age.

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

All mice used under this license will be monitored for signs of pain and distress and inspected frequently for clinical signs, which will be recorded on score sheets. This will take account of things like appearance, posture, and behaviour. Tumour size will also be inspected, palpated, and measured by callipers and recorded. Mice weights will also be monitored and recorded.

If any clinical signs are found, mice will be promptly treated wherever possible (for example, with the use of different diets, analgesics for pain relief, and/or antibiotics), and if suffering cannot be promptly alleviated, mice will be humanely killed.

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

Much of the guidance we use is based on our experience of our mouse lines, but also on the best practice resources for genetically altered animals on the NC3Rs website (https://www.nc3rs.org.uk/breeding-and-colony-management). We also seek advice from our NVS, NACWO, and the animal technicians in our facility who have regular contact with our animals. The animal experiments in our papers are described in accordance with the ARRIVE guidelines. The guidance documents and Position Papers from LASA on Transgenics, Education and Training, the 3Rs and the Concordat on Openness and Workman et al., 2010, *Guidance for the welfare and use of animals in cancer research*, are all relevant to my work.

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

I am registered for monthly updates from the NC3Rs website, which is a major resource for advances in the 3Rs and can consult the Laboratory Animal Science Association (LASA) guidance documents. I will also be in regular contact with members of my Project Support Team, which includes my NVS (Named Veterinary Surgeon), NACWO (Named Animal Care & Welfare Officer) and NIO (Named Information Officer). I can also consult resource pages on the NC3Rs website (https://nc3rs.org.uk/resource-hubs) and 3Rs in-house search tools and Norway's National Consensus Platform for the advancement of the 3Rs (Norecopa: https://norecopa.no/databases-guidelines), an external resource that maintains 10,000 pages of information and searchable databases about 3Rs strategies.