Home Office

NON-TECHNICAL SUMMARY

The role of ubiquitin ligases in disease and the development of new therapies.

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

neurodegeneration, male infertility, cancer, protein homeostasis, therapeutics

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

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?

This project aims to understand the function of a ubiquitin ligases in different cell types, investigate how their malfunctioning leads to different diseases, and test their potential as targets for new treatments.

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 ligases are essential enzymes (proteins that help speed up chemical reactions in our bodies) that regulate a wide range of cellular processes critical for cell health and function. The dysregulation of these proteins is a fundamental cause of many human diseases, including cancer, Parkinson’s disease (a neurodegenerative disorder that affects movement), and infertility. This project aims to investigate the specific functions of important ubiquitin ligases to understand how their malfunction contributes to disease. Given their highly specific and influential nature within the cell, we will explore their potential as targets for new treatments designed to reduce or cure these conditions.

Cell and computer-based studies are useful and will be used wherever possible, but they cannot show how these proteins work across the whole body. The effects of ubiquitin ligases often depend on interactions between different tissues and organs, and on processes such as development, reproduction, brain function, and disease progression. These cannot be fully reproduced outside a living animal.

Mice are needed because they allow us to study these proteins in a whole-body system, and because many of these proteins work in similar ways in mice and humans. This will help us better understand disease and support the development of future treatments.

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

This project will produce several important outcomes. Most importantly, we will gain new understanding of how certain key proteins control vital cell functions, such as energy production and protein recycling. We will also learn how problems in small groups of cells can affect the health of the whole body. Using this knowledge, we will test new treatments designed to target these protein pathways in disease models. We expect this work will result in scientific publications and discoveries that could be developed into new treatments. The ultimate goal is to build a solid foundation of evidence that will help design future clinical studies for patients with conditions like Parkinson's disease, male infertility, and cancer.

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

Downloaded: 2:43pm, 13 May 2026

The main beneficiaries of these outputs will be the scientific and clinical communities focused on diseases like cancer, Parkinson's disease, and male infertility. To put this in context, approximately 145,000 people in the UK are diagnosed with cancer each year, around 120,000 people live with Parkinson's disease, and male infertility affects about 1 in 20 men. In the short term, our publications will inform new research directions and drug discovery efforts, while also providing clinicians with a deeper understanding of the underlying causes of these conditions. The long-term objective is to translate this fundamental knowledge into the design of new treatments for patients.

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

We will maximise the outputs from this work by sharing results quickly and widely. We publish in Open Access journals, post preprints on bioRxiv, and deposit research datasets in public repositories so that other researchers can find and use them. This includes sharing negative and unexpected findings where appropriate, to help avoid unnecessary duplication of work.

We will also share resources generated during the project. For example, plasmids will be made available through Addgene, and proteomics and metabolomics datasets will be deposited in public databases such as PRIDE. Tissues will be shared with other researchers through local and collaborative arrangements, including the [redacted]. Unique mouse lines will be preserved by sperm cryopreservation and can be archived, for example at [redacted], or shared with other researchers on request. We will also share methods, protocols, and expertise through publications, conferences, and collaborations.

Species and numbers of animals expected to be used

- Mice: 3010

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 use mice to study what happens when an important cellular protein is lost in complex body tissues, because these studies cannot be done using alternatives to animals. Mice are the most appropriate species for this work because they are a well-established mammalian model, many of the genes and biological pathways we are studying are similar in mice and humans, and there are established genetic tools that allow us to alter specific genes in a targeted way. This means we can answer the research questions in the most suitable species without the need to use larger animals.

For example, understanding how sperm develops requires the complete structure of the testes of a sexually mature male mouse at about 10 weeks of age. The final steps of maturation from a round sperm cell into the elongated shape with a head and a tail is a process that cannot be recreated in a laboratory dish, so we have not done any *in vitro* studies of sperm development. Adult animals are

required here because these stages only occur after sexual maturity, when the testes are fully developed.

We are also studying progressive brain diseases similar to human Parkinson's disease, which occurs in people with changes in a specific gene and the biological pathways it controls. Our mouse model closely mirrors the key features of this human disease, showing a gradual, age-related loss of brain cells that leads to worsening movement problems over time. This complex disease process, which develops slowly as the animal ages from 6 weeks through to 30 weeks of age, requires the brain structures found in mammals and either does not occur or works very differently in simpler animals. Older animals are needed in these studies because the disease changes develop progressively over time and would not be seen fully in younger mice.

We have undertaken *in vitro* studies differentiating human stem cells into the vulnerable nerve cell type that dies in Parkinson's disease patients to undertake biochemical studies or functional imaging of live cells in a dish. We use these non-animal methods wherever possible as part of our 3Rs approach, to answer specific molecular and cellular questions before moving to animal studies. However, these do not reflect all the cell types involved or the complex architecture of the midbrain, and they cannot model the gradual, age-related progression of this degenerative disease. For these reasons, studies in mice remain an essential and unique tool for understanding the causes of these diseases.

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

To meet our research goals, mice will be specially bred to carry specific changes in important cellular proteins, creating animal models of diseases like male infertility and progressive nerve cell death. The breeding process requires regulated procedures including injections for hormone treatment, surgical procedures such as vasectomy to create sterile males, embryo transfer procedures, and tissue sampling for genetic testing. Male infertility work will all be done on post-mortem tissues.

About 100 of these animals used for studying progressive nerve cell death will receive a single injection into a vein containing a virus that carries a healing gene, and some animals will undergo injections with a radio-labelled tracer to enable imaging of nerve cell function to track the treatment effects. This treatment is designed to slow down or stop the brain cell death that would otherwise occur over 5-30 weeks.

In separate cancer research, up to 100 mice will be injected with cancer cells under the skin to grow tumours. Once these tumours can be felt, about half of these animals will receive injections of new treatments designed to slow or stop tumour growth, while the other half will serve as comparison groups. These cancer experiments typically last 4-12 weeks.

At the end of all studies, the animals will be humanely killed using different methods depending on the tissues needed - some will be killed by injection of overdose anaesthetic, others by dislocation of the neck or by carbon dioxide exposure. These different methods are carefully chosen to ensure the best preservation of tissues, especially the brain and reproductive organs, for essential laboratory tests afterwards.

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

Downloaded: 2:43pm, 13 May 2026

The majority of animals used on this licence are expected to experience only mild adverse effects. One of the strains of mice has only a reduction in the expression of a ubiquitin ligase in its tissues, and it shows mild phenotypes, including an anaemia in all mice and males are also infertile. Male infertility means the mice cannot produce offspring but otherwise behave normally. The anaemia is mild and is present in both sexes, and it is due to lower overall numbers of red blood cells produced at the last steps in their maturation, similar to the sperm cells. In people, mild anaemia can cause tiredness and reduced activity levels, but these mice behave normally. Some mice with complete loss of the expression of the ubiquitin ligase in one type of nerve cell in the brain may develop problems with walking and standing due to progressive loss of brain cells. Injections may cause brief discomfort at the injection site. In our cancer therapy studies, mice will develop tumours under their skin that may cause discomfort, weight loss, and reduced activity as they grow. We will test the effectiveness of new treatments we are developing to reduce or halt 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 41%

Moderate 59%

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

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?

We use non-animal methods wherever possible as part of our replacement strategy. This includes experiments with human cells, patient-derived cells, stem cell-derived nerve cells, and laboratory-based biochemical studies. These methods are used first to answer molecular and cellular questions and to make sure animals are only used where there is no suitable alternative.

However, some of the main aims of this project cannot currently be achieved without the use of animals. We need to understand how faults in certain ubiquitin ligases affect whole tissues and lead to disease over time. For example, the final stages of sperm development depend on the structure of the

testis and on close interactions between different cell types, which cannot yet be recreated fully in a dish. In our studies relevant to Parkinson's disease, we also need to investigate how changes in a specific gene cause the gradual loss of nerve cells and movement problems as the disease develops with age. These long-term changes depend on interactions between many cell types within the organised structure of the brain and cannot be modelled adequately using current non-animal methods.

For this reason, animals are used only for the specific unanswered questions that require a whole living system, and only after non-animal approaches have been used as far as possible. We will continue to keep this under review throughout the project and will adopt validated alternatives if and when they become available.

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

Before using any animals, we do as much of the work as possible in the laboratory and use non-animal methods wherever they are suitable for the question being asked. To find possible alternatives, we keep up to date with published research by searching databases such as PubMed, and we also use guidance and resources from the NC3Rs, EURL ECVAM (for their non-animal models of neurodegenerative diseases) and the Norecopa 3Rs Guide. This helps us identify methods that may be able to replace or reduce the use of animals.

For example, we use stem cells to make nerve cells in the laboratory, including 3D structures called organoids that better reflect the organisation of human tissue. We also work with skin cells donated by patients, which can be converted into nerve cells in a dish so that we can study how those cells are affected by disease. In addition, we use gene editing to introduce disease-causing changes found in patients into stem cells. This gives us a reliable supply of specialised nerve cells for testing and helps reduce the number of genetically altered mice that need to be created.

We also consider other non-animal approaches where they may be useful, including human cultured cell lines, tumour cell culture models, ex vivo methods, computational approaches, and other laboratory-based biochemical and imaging studies. Wherever possible, these are used first for mechanistic studies and early-stage testing.

Together, these approaches allow us to answer many questions without using animals and help ensure that animals are only used when non-animal methods cannot provide the information needed.

Why were they not suitable?

These non-animal methods are very useful and are already used for a large part of the project, but they cannot yet answer all of the specific questions we need to address.

For the neurodegeneration work, human stem cell-derived nerve cells and organoids are helpful for studying early molecular changes and the vulnerability of the affected cell type. However, they do not reproduce the full organisation of the brain, long-range connections between brain regions, or the gradual, age-related loss of nerve cells that leads to changes in movement over time. Because one of our aims is to understand how these changes progress and affect function in the whole animal, these methods are not sufficient on their own.

Downloaded: 2:43pm, 13 May 2026

For the fertility work, cell-based approaches cannot recreate the full structure of the testis or the close interactions between the different cell types that support sperm development. One of the aims of this project is to understand how changes in specific proteins affect the final stages of sperm shaping and maturation. These stages depend on the normal environment of the intact testis and cannot currently be modelled at all *in vitro*.

For tumour biology, cell culture models are useful for studying cancer cells themselves and for testing some molecular mechanisms. However, they do not fully reproduce the tumour environment, including the interactions between tumour cells, surrounding healthy tissues, blood supply, and immune cells. Where the scientific question depends on these interactions, non-animal methods are not enough by themselves.

Animal use is therefore restricted to these specific unresolved questions, where a whole living system is needed to study how disease develops and affects tissues and function over time. We will continue to use non-animal approaches wherever they are scientifically suitable, and we will keep new alternatives under review throughout the project.

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?

Animal numbers have been estimated from a combination of breeding expectations, previous experience with these models, pilot studies, and the size of experimental groups needed to detect meaningful differences between groups. For some of our genetically altered mice, several genes must be inherited together, so only a proportion of pups born will have the required genetic change. For example, in the Parkinson’s disease studies, approximately 1 in 8 pups will lack the target protein in neurons. We therefore estimate numbers based on around 5–6 breeding pairs producing 5–6 litters of around 5–6 pups, which should generate enough animals for experiments while avoiding unnecessary overbreeding. In the fertility studies, around 1 in 4 pups will have the required genetic change, and only half of these will be male, so around 3 breeding pairs should be sufficient.

Experimental group sizes are based on pilot work, previous studies, and the minimum number needed to allow comparison between treated and control groups. For example, for virus treatment studies we estimate needing around 100 mice based on pilot studies, and for tumour studies we estimate around 100 mice, with half receiving the treatment and half acting as controls. Control groups are included where needed to make sure the results allow us to distinguish treatment effects from normal biological variation.

We reduce variability by using well-characterised, published mouse strains, standardised breeding and housing conditions, consistent experimental procedures, and by comparing animals of the same sex

Downloaded: 2:43pm, 13 May 2026

and similar age wherever possible and appropriate. This helps reduce the number of animals needed to draw reliable conclusions.

We also aim to maximise the information obtained from each animal. Wherever possible, more than one type of analysis will be carried out from the same animal, for example combining clinical observations with post-mortem tissue analysis.

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

We have studied these specially bred mice for 10 years and have found the clinical signs to appear in 100% of the mice with the relevant genetic changes. This predictable outcome reduces variability and means we need fewer mice to generate statistically meaningful data, because we do not need to account for uncertainty about whether the disease will develop.

To reduce bias and make the data more reliable, we use randomisation (randomly assigning mice to different treatment groups) and blinding (researchers do not know which treatment or genotype each mouse has during assessment) wherever possible. We also control known sources of variability by using animals of the appropriate sex, similar age, and consistent genetic background, and by using littermate or age-matched controls where possible. Animals are also housed, handled, and assessed under standardised conditions to reduce unnecessary variation.

Our experimental design is informed by our previous work with these mouse lines, pilot studies, and published guidance. When planning new studies or introducing new interventions, we follow NC3Rs guidance and use formal design tools such as the Experimental Design Assistant where appropriate. We also stage experiments where possible, so that early findings can be reviewed before larger studies go ahead. This allows stop/go decisions to be made and helps ensure that no more animals are used than necessary.

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

In estimating numbers for rAAV and tumour model studies, 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.

Efficient breeding means carefully managing our breeding colonies by replacing breeding pairs after 6 litters, replacing pairs with small litter sizes or care issues, and timing breeding to minimize the number of animals produced. We will perform pilot studies testing any new therapies in very small groups of normal mice first. 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 and minimize the suffering of animals.

Downloaded: 2:43pm, 13 May 2026

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 will use genetically altered mouse models in which the ubiquitin ligase we are studying is either reduced throughout the body or disrupted only in specific cell types, such as neurons or germ cells in the testes. Wherever possible, we use these more targeted models to restrict the effects to the tissues of interest and avoid unnecessary illness.

We considered non-animal alternatives, including cell-based systems such as human stem cell-derived nerve cells, organoids, and other laboratory models, and we use these wherever possible. However, they cannot reproduce the full complexity of disease developing over time in a whole living body, or the interactions between different cell types and tissues that are needed for this project.

More severe alternatives, such as complete gene loss in all tissues or more aggressive disease models, were considered but are not used unless scientifically necessary, as they would be expected to cause earlier or more severe effects without providing extra scientific benefit. In our cancer studies, we will use well-established tumour models in which tumours are grown under the skin, rather than more invasive internal tumour models, to allow treatment response and disease features to be studied with less harm.

These models have been chosen to keep pain, suffering, distress and lasting harm as low as possible while still allowing the scientific aims to be met. Further reduction in the duration or intensity of effects is not possible where disease progression or treatment response must be followed over time.

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

We can’t use immature forms of mice because the neurological signs we are studying develop over time, requiring mice to live from 3 weeks to 30 weeks of age. Additionally, male mice only become sexually mature at 10 weeks of age. We can’t use less sentient animals like flies or worms, because they don’t contain the genes we study. Higher vertebrates like fish and frogs don’t have the similar brain structures as mammals. We can’t use terminally anaesthetised mice because we need to observe how the diseases progress over weeks and months.

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 handled using non-aversive (gentle) techniques and will be allowed to acclimatize to their environment to reduce stress. Mice are not handled unnecessarily. Bedding is cleaned out once a week, and enrichment is added, like chewable bedding for warm nest construction, or a plastic box or tube for hiding/privacy. The radio is kept on during working hours in the facility, and light and darkness are controlled automatically on a 12-hour alternating cycle. Individually ventilated cages are kept at a constant temperature and with filtered air. Mothers with new litters are not disturbed soon after giving birth.

Our mice have continuous access to food and water, and occasionally wet mash. Cage occupancy is monitored to ensure mice are housed with companions, whenever possible. Females are consolidated at any age, and males are combined up to 5 weeks of age. Occasionally, stud males are housed alone if awaiting an appropriate genotype female to be paired with or if fighting or if they show signs of ill health. Animal technicians will report if any animals are alone in a cage. Pups are not ear notched until approximately 2 weeks of age.

Procedures are carried out by trained technicians or trained members of our group. Anaesthesia and, where appropriate, analgesia will be used for all surgical procedures to prevent or minimise pain, and single-use needles are used to reduce the risk of infection. These measures are intended to reduce the severity of procedures as far as possible.

All mice will be monitored for signs of pain and distress so that problems can be identified early and action taken quickly. We inspect mice frequently and record clinical signs on score sheets, monitoring things like appearance, posture, movement, and behaviour. We also monitor body weight, because weight loss can be an early sign of illness or distress. Tumour size will be measured using callipers, which is more accurate than visual assessment or palpation and helps us identify early when tumour volumes are approaching the allowed limit.

Humane endpoints will be applied as soon as animals show clear signs that they may be experiencing moderate pain, suffering or distress, rather than waiting for signs to become more severe. This means that where clinical signs develop, mice will be treated promptly wherever possible, and if suffering cannot be quickly relieved, or if predefined humane endpoint criteria are reached, mice will be humanely killed without delay. Regular monitoring and early intervention are used throughout the project to minimise welfare harms as far as possible.

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. We have given a talk to the technicians to explain the science behind our experiments and to discuss their experience 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, will be used in my work. For tumour models, the OBSERVE guidelines that have been tailored to reflect the work carried out at UBS will be

Downloaded: 2:43pm, 13 May 2026

followed: Observe Guidelines - Cancer Models Guidance | University Biomedical Services (UBS) https://www.ubs.admin.cam.ac.uk/observe-guidelins-cancer-models-guidance

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 am also 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 will 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.