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NON-TECHNICAL SUMMARY

Models of mitochondrial diseases: pathogenesis and therapy

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Mitochondrial disease, Model generation, Gene therapy, Experimental therapy

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?

To generate and maintain mouse models to understand mitochondrial diseases and and develop effective 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?

Mitochondrial diseases arise from harmful changes (i.e. mutations) in mitochondrial and/or nuclear DNA, which produce molecules important to normal cell function. These mutations cause changes in the structure and function of mitochondria, which disrupt the normal mitochondrial processes such as energy production (via oxidative phosphorylation), mitochondrial replication and removal (via fission and fusion), and ion transport across the mitochondrial membrane (important for normal cellular functioning). Impairments in one or more of these mitochondrial processes present in patients as diverse metabolic disorders which are difficult to treat, due to the wide range of possible signs and symptoms of these diseases. The work proposed within this licence aims to understand the mechanisms underlying mitochondrial diseases and develop more targeted treatments (such as pharmacological and non-pharmacological therapies).

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

From the work undertaken in this project, we expect three main outputs:

1. New mouse models of mitochondrial disease which carry mutations similar to those found in humans. At present, there are only a handful of mouse models which replicate the signs and symptoms experienced in patients with mitochondrial disease. More models are required to support outputs of points 2 and 3.

2.The molecular, cellular, and behavioural characterisation of the resulting models, that will grow our understanding of mitochondrial function and inheritance both in health and disease.

3. Potential treatments for correcting the dysfunctional mitochondrial in mice and provide initial data for future clinical translation. At present, there are no cures for mitochondrial diseases.

Outputs from this work will support future applications for funding and potential patents. Outputs will be shared with other researchers in the field via publications in scientific journals and presentations at scientific meetings.

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

In the short and medium term, the models generated will be used to expand our knowledge on the basic mechanisms of mitochondrial biochemical pathways and the impact of altered gene states in the execution of these pathways. The models developed will be characterised and samples will be collected and made available to internal research groups and external collaborators. The knowledge gained will be used to develop and test of novel therapeutic interventions. This is done with the long-term view of opening new avenues of therapy for patients with mitochondrial diseases.

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

This output of this work will be shared with both national and international collaborators, as well as published in relevant scientific publications.

Species and numbers of animals expected to be used

- Mice: 22675

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.

Mice have the closest genetic relationship of a model organism to humans. They have a short lifespan which allows researchers to study animals throughout development and adulthood within a relatively short time. In addition, mice are prolific breeders. Over recent decades, the genetic material of mice have proven to be manipulatable using gene-editing technologies.

Mitochondrial diseases can occur at any age. Therefore, we propose using animals across the life stages, from embryo to adult, to study dysfunctional mitochondria, depending on the hypothesis being tested (e.g. inheritance of mutated mitochondrial DNA or accumulation of mutations during adulthood).

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

Substance administration: Animals used in this project may undergo injection of chemical and/or biological substances, with or without anaesthesia, surgical procedures, sampling of blood/cells/tissues on multiple occasions, and/or administering drugs via food or drinking water. Substances may be given by injection to the eye, vein, muscle, or directly into the brain.

Behavioural testing: Animals used in this project may undergo behavioural testing [e.g. testing muscle function using a treadmill test and/or testing the function of the eye using electro-oculography (EOG), etc.] to characterise the functional differences resulting from the mutation in the mouse mitochondria.

Animals may undergo a combination of both substance administration combined with behavioural testing.

The majority of animals will undergo five or less regulated procedures during the course of an experiment.

It is estimated that the majority (approx. 80%) of animals undergoing regulated procedures will be juvinelle (up to 8 weeks old) and adults (more than 8 weeks old). The remaining 20% will be neonates (new born pups up to weaning - approx. 3 weeks old).

Experiments will vary in duration from days to months. At the end of breeding or experiments, animals will be humanely killed and tissues collected for further study.

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

Due to the disruptions in metabolism caused by the gene mutations and/or the therapies applied, some animals may experience symptoms such as weight loss, abnormal movement (e.g. uncoordinated gait), altered behaviour, and possible pain and discomfort. In most cases, pain, stress and discomfort will be kept to the minimum and, if present, it will be mild and transient. Where stated in the licence, pain will be alleviated by giving analgesia. Animals exhibiting behavioural and motor differences may suffer Moderate severity due to the impact of these symptoms on their normal behaviours in the cage, including feeding. Animals experiencing Moderate severity will be closely monitored for weight loss (to gauge the impact of the abnormal movement on their ability to feed) and will be killed immediately if >20% of their baseline body weight is lost. In most cases, weight loss to this degree is observed over weeks. Animals that display ataxia (i.e. observed as a wobbly, lurching gait) for more than 24hrs will be killed immediately.

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:	Sub-threshold	33.3%
	Mild	57.27%
	Moderate	9.43%

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?

Animal experiments remain a necessary part of our research as mitochondrial diseases affect multiple tissues in the body (e.g. brain, muscle, liver). In order to develop mouse models that mimic human patients with mitochondrial disease, the whole animal is required to monitor for signs of disease. This multi-system affect cannot be replicated *in vitro* (i.e. in isolated cells in the lab).

In addition, the gene therapies we develop need to be tested for target specificity when delivered systemically (i.e. administered into the blood via injection of a vein). This currently cannot be simulated *in vitro* and is a vital step towards translation of our novel therapies to treat patients.

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

Mouse adult fibroblasts (MAFs) and mouse embryonic fibroblasts (MEFs) may be harvested and used to generate cells which can be studied *in vitro*, reducing the number of animals used in some cases. Prior to testing in animals, gene editing therapies are tested *in vitro*, using established mouse cell lines, for their efficacy prior to testing *in vivo* (i.e. in a living animal, in this licence the animals used are mice).

Why were they not suitable?

The study of cells *in vitro* does not simulate the environment of the entire body. Hence, *in vitro* cell culture can help to refine treatments prior to *in vivo* testing but cannot replace the interaction of all the tissues at once, as in the whole animal. Cells cannot be used to study animal behaviours which may be perturbed in mitochondrial diseases, for example walking, feeding, or vision.

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?

For mitochondrial disease models, the estimated animal numbers are based on our past experience, and reflect the challenges of maintaining a colony of female mice transmitting mitochondrial DNA (mtDNA) within the desired ranges. The effect of unknown mtDNA selection during gestation contributes to the instability of mutation levels in a mouse litter. Maintaining mutation levels within the desired experimental range requires a larger number of breeding pairs due to changes in mutation level during transmission.

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

The PREPARE guidelines are considered prior to experiments, as well the NC3R's Experimental Design Assistant. These guidelines include calculation of required sample size for the chosen analysis method and careful planning of colony expansion to ensure any surplus of animals generated is kept to a minimum. Where possible (e.g. animals of appropriate age and sex are available) we will source wild type animals from the 3Rs enquires list for this project.

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

Colonies will be reviewed routinely and their size adjusted according to experimental need. Wild type mice will be bought in when needed, removing the requirement for keeping a wild type colony.

Pilot studies will be conducted for planning complex dosing, that require initial *in vivo* validation and optimization.

In the majority of experiments, it is not optimal to randomise the animals selected for treatment vs control groups. In most cases, we want to study the animals with the highest levels of mutant DNA. Blinding is possible at the point of administration (e.g. substance administration), however blinding may not be effective due to the resulting changes in the animals health or behaviour during the course of the experiment.

We will use a superovulation protocol to increase embryo yields. This results in a reduction in the number of females used, as we can collect more embryos for study. In addition, this will allow the colony to be kept smaller as we will not require as many females with the desired genetic profile.

Whenever possible (e.g. animals of appropriate age and sex are available), we will endeavour to source wild type animals from the 3Rs enquires list.

Whenever possible, we will co-ordinate internally to make spare animals/tissues available to researchers within our department. In addition, we are listed on a 3R's enquiry list, where we shall list surplus tissue/animals for other researchers to use.

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.

Mice will be used to model human mitochondrial diseases. The majority of these mice display mild symptoms of diseases. Mice are the least sentient animals available that share very similar anatomy and mitochondrial functions as humans.

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

The life stages used under each protocol have been carefully considered and meet the experimental life stage need (e.g. early interventions for mitochondrial diseases need to be given to neonatal pups, as therapies may be more effective early in life).

Mice are good breeders, producing offspring with the desired genetic profiles in a feasible time span to allow for experiments to be conducted within the time frame of the licence. Live animals are required to test *in vivo* therapies. Wherever possible, animals will be killed prior to tissue harvest.

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

Animals undergoing substance administration will be monitored daily, in some cases twice daily (AM and PM). Following a regulated procedure, a post-procedure welfare check will be scored using a procedure-specific check sheet, to assess the body condition, feeding behaviour, and (e.g.) site of administration. The procedure-specific score sheets will be developed with the advice of the NACWO and/or NVS. Adherence to the welfare check sheet will ensure that the humane endpoints are adhered to and the assigned severity levels are not exceeded.

Where required, peri-surgical analgesia (i.e. pain relief) will be administered to animals, with the use of voluntary treatments such as medicated flavoured jelly, paste, or milk shake liquid.

All users are trained and encouraged to use methods of handling of the animals to reduce stress (e.g. hand cupping or use of tunnel).

Wherever possible (i.e. in the majority of cases), animals will be group housed. In the minority of cases, it may be unavoidable that males are singly-housed. For example, stud males cannot be group housed due to fighting, which may cause greater suffering than single-housing. In addition to the group housing of animals, enrichment of the home cage will include suitable size cages for the animals age/size, including contents such as tunnels and nesting material, as well as a mezzanine level and freely available food and water.

Where the genetic mutation affects the ability of the animal to feed, for example due to altered behaviour/motor impairments, feeding may be assisted by providing water gel, food, and/or mash diet on the cage floor.

All wild type animals brought into the animal facility will be held for 15 days acclimatization and health monitoring before undergoing a regulated procedure.

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

The following guidelines are referred to and considered prior to starting new projects:

- The PREPARE guidelines, which consists of planning guidelines for *in vivo* experiments, will be considered prior to starting experiments.
- LASA (Laboratory Animal Science Association) guiding principles, which provide up-to-date guidance on training and education for PIL holders, aseptic surgical practices, good record keeping.
- RSPCA guidance on Research Standards and the role of the 3R's in improving the planning and reproducibility of animal experiments.
- National Centre for the 3Rs (NC3Rs) resources on experimental design, genetic modification, handling, husbandry, welfare assessment, and *in vivo* techniques.
- Breeding resources from sources such as Jackson Laboratory, The International Mouse Phenotyping Consortium (IMPC), International Society of Transgenic Technologies, and the International Mouse Strain Resource (IMSR).

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

The PPL holder and PIL holders associated with the licence are subscribed to the 3R's enquiry email list. The Named Information Officer sends regular emails with updates on regulatory guidelines and available resources. In addition, the PPL holder routinely attends events organised by the NC3R to keep up to date with the latest developments in animal welfare, experimental design, and new guidelines. The PPL holder attends national and international conferences on animal welfare and the use of animals in scientific research (e.g. Federation of European Laboratory Animal Science Associations 2022 conference in Marseille, France).