NON-TECHNICAL SUMMARY

Therapeutic development for sphingolipid diseases

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.
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

Genetic, disease, neurodegeneration, cancer, sphingolipids

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project’s objectives, for example the scientific unknowns or clinical or scientific needs it’s addressing.
What's the aim of this project?

The applicant takes care of patients suffering from a rare family of inherited, relentless and fatal diseases all of which affect the nervous system but may have systemic features (called the sphingolipidoses). These diseases affect all ages but are very severe in babies and children.

The individual diseases called (Gaucher disease, Krabbedisease and GM2 gangliosidosis – Tay-Sachs and Sandhoff diseases) all affect the way the body recycles complex fatty molecules mainly in the brain. Cancers also occur in patients with Gaucher disease – which giveshas provided a clue about the cause and possible treatment of such cancers in the general population. We have recently found that identical cancers arise in the mouse model of Gaucher disease.

The overall aim of this application is to generate the data necessary to expand understanding of the fundamental processes which result in sphingolipid disease and use this data in the development of 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.

What are the potential benefits that will derive from this project?

We will advance clinical treatment of a group of closely related but cruel and relentlessly progressive hereditary defects in the brain and other tissues by directly and safely restoring the defective functions. We will explore corrective gene transfer as well as medication also that has promising actions to improve the outcomes for patients who will otherwise die and endure extreme distress and disability – for there are no effective cures. We will also apply these techniques to test in detail whether in one condition (Gaucher disease) we will be able to prevent associated cancers - we already have evidence indicating that this may be possible and to justify possible recommendations in patients, need to find out exactly how this therapy might work.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will only use mice and plan to use less than 27000 over five years. We appreciate that this is a large estimate over the period but now it must reflect not only the minority of animals undergoing procedures to test treatments and work out what exactly causes the disease, but also the much greater number of healthy animals which have traits that are required for the complex breeding purposes to obtain those for special study in the particular diseases. These will either not suffer or have only mild effects from the breeding. An additional reason for the much larger number than formerly estimated is the expected requirement to move to improved facilities in 2019: this will require re-derivation of the strains for safety and health management and establishment in the new facility.

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Partial treatment or belonging to a control group with no active treatment (as required for valid research studies) may lead to mice with genetic diseases living longer with signs of muscle weakness, tremor and weight loss. We will monitor our animals very closely to ensure that their well-being and capacity to fend for themselves is impaired as little as possible and that there is no expectation of pain or sign of distress. A proportion of animals will develop tumours of the blood (lymphoma or myeloma) which is observed in human patients with Gaucher disease. Cancer prone animals (less than 15% of the total number of animals used) will be closely monitored and killed humanely before they develop clinical signs of lymphoma and myeloma (which include enlarged lymph nodes, anaemia [pale paws and ears], loss of body condition, muscle weakness and reduced exercise tolerance). In all cases where signs of pain or where animals start to show behavioural signs, difficulty moving due to muscle weakness or signs of weight loss or body condition will be killed immediately using a humane method. The licence will allow the breeding and maintenance of mice, some of which may develop muscle weakness, tremor and tumours. In addition, most animals (approx. 7650) will be used in experiments where animals will receive treatments, blood sampling and in some cases non-invasive imaging (such as MRI) and exposure to irradiation (in less than 10% of all animals). Some of these procedures may require anaesthesia (for example surgical interventions to inject into the brain) or for restraint (for example imaging and irradiation). Whenever surgery is performed, animals will receive pain relief treatment. In addition, the surgeons who work under this licence have the necessary experience to perform the procedure causing minimal suffering on the animals. The treatments tested under this licence will either improve the health of the animal or have no effect. All animals are killed humanely at the end of the experiment. Most of the animals used in the licence will be used to obtain those with disease traits but will not show disease themselves; their use in breeding must be noted but the effects of procedures on them will be mild. The work reflects a complex design and it has been challenging to fit this into the new licence format. The revised numbers now include all the experiments envisioned: all are of direct medical significance and/or importance for the severe and life-shortening diseases we study and in patients for whom we provide care. Finally we justify the modified application because these diseases mainly affect young adults and children whose great clinical needs cannot at present be provided for, because the research is closely linked to the discovery and application of specific treatments and because at this stage there is no alternative to the use of animal models.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Where possible we conduct appropriate signalling and in vitro studies using human blood cells. Normally, these are undertaken as confirmatory studies of molecular mechanism but unfortunately only contribute incomplete information because of the ethical constraints of human clinical research.
It is too early to test all the treatments such as gene therapy in patients and we have models in mice that very closely resemble the human condition. Here the diseases can be more safely and comprehensively studied.

There are no alternatives for decisive testing at this stage but we will use samples from patients as soon as possible - and also do experimental medicine in human patients rather than animals as soon as we have the confidence (and permission) to do so.

Reduction

**Explain how you will assure the use of minimum numbers of animals.**

From previous development we know that our proposed treatments should have big effects when used in the correct way: we will design the research to give clear results which can be examined in great detail to minimize the numbers of animals that would have to be used to test our expectations for human use. Before carrying out regulated procedures, the experimental plan will be analysed using suitable power estimations.

We have long experience of, and access to biostatisticians who will advise on the design of our studies to ensure that suffering is kept to a minimum for the least number of animals according to guidelines; proper controls will be used to add confidence to the findings. For investigations into treatment and cause of disease, mating will be optimised: where we mate young mildly affected mice and carriers to obtain twice the yield of those required that model the disease and reduce production of healthy unneeded animals. the most mice that model the disease so that there is minimal waste of animals.

For very rare animals needed to test the potential effects of modifying traits, we will use the new CRISPR-cas9 technology that allows models to be bred efficiently with the disease traits using a few mice and only two or three rounds of breeding. To minimize the chances of passing on unwanted random traits induced by this technology, the first animals obtained with the desired trait will be bred with their parents rather than interbreeding from siblings. CRISPR This will minimise the number of breeding cycles required and will reduce the risk of transmitting unintended genetic defects to the offspring.

After collection, the data will be analysed by appropriate statistical methods that meet internationally recognised standards of research practice and experimental design set out in the Animal Research: Reporting of In Vivo Experiments (ARRIVE) NC3Rs guideline documents (National Centre for the Replacement Refinement & reduction of Animals in research -www.nc3rs.org.uk/ARRIVE).

Strong beneficial effects of our treatments or genetic factors in the disease reduce the number of animals required to evaluate the effectiveness (or toxicity) in study designs. Fortunately, small focussed study groups usually prove adequate for us to obtain clear information so that repetition will not be needed.

Refinement
Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

There are several models of the severe diseases we study in animals; however we use mice which have exactly the same genetic problems that cause the human conditions. These mice will be the smallest and least feeling or threatened and distressed animals we can find usefully to test; and we plan to study them under strict legally enforced surveillance and regulations.

To get clear and efficient results, we plan only to do the minimum work on small numbers in each group of mice; so far as possible we make point of using all the materials and cells obtained from these animals.

Every effort has been put into the design and humane conduct of this work and we will be observing the animals to be sure that any suffering is kept mild or at the most moderate and for as short a period as is required to be informative.

There are no alternative experimental models of Gaucher disease and our mice develop the very same cancers that occur in human patients. For the disorders of the brain, previous studies in cats, sheep, dogs and monkeys have been done by American collaborators built on our research. Now these are reported, we will carry out licensed procedures only in mice to discover the basic causes of the diseases as well as the information needed to support the delivery of new medicines (a tablet for the cancer and a gene therapy for the brain disorders in children).

In the cancers, related to Gaucher disease (lymphoma, myeloma and rare liver tumours), we will predict their onset with signs in the blood that anticipate development of full-blown cancer. These will prevent unnecessary suffering to the animal well in advance – as in patients. No animals will be kept until they would die of the disease. Instead they will be carefully monitored using a pre-tested clinical score and killed at an earlier stage to minimise suffering.

Our procedures are well-tolerated: mice make a quick recovery and care is taken to keep them warm with access to food and water during convalescence. With long experience, humane endpoints have been developed; we recognise that high-level monitoring may be needed to avoid distress. When drugs are used: substrate reduction therapy and chaperones, anti-inflammatory agents, and immune-suppression protocols, doses will be based on published data, veterinary, and our experience. We will not develop drugs that require primary testing in mice, since pilot studies will have been already done by our collaborator. Agents with significant side effects (e.g. weight loss) will be used at low doses in the first instance; and substitutions will be actively explored. To minimise transient loss of appetite (side effects of interferon inducer poly IC), softened food or sweeteners may be placed in the cage floor at pre-weaning stage.