Home Office

NON-TECHNICAL SUMMARY

Developmental and degenerative mechanisms in ciliopathies

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 following aims mentioned in paragraph (b)

Key words

Ciliopathies, Retinal degeneration, Obesity, Bardet-Biedl Syndrome, Gene therapy

Animal types	Life stages
Mice	neonate, juvenile, 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 is the aim of this project?

Our work focuses primarily on a group of human genetic disorders known as ciliopathies, including Bardet-Biedl Syndrome (BBS). This project uses genetically mutated mouse lines to understand and study the biological mechanisms that cause defects and organ failure in these type of disease.

These conditions have no cure. Here we also aim to develop new technologies, such as gene therapy and stem cells solutions and test their efficacy and safety on our mouse mutant strains. In order to tackle the different aspects of these disorders we will deliver our treatments using different routes of administration at different stages of the 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?

The final goal of this project is to prepare the first in human clinical trials. For this we will execute the Proof of Principle and Clinical Trial enabling applications.

The work we will develop will be the base for the pre-clinical reports necessary to approach regulatory bodies, such as the UK Medicines & Healthcare products Regulatory Agency (MHRA), the European Medicines Agency (EMA) or the U.S. Food and Drug Administration (FDA).

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

The ultimate goal of our research is to make fundamental discoveries on the causation, mechanisms and prevention and treatment of ciliopathies and Bardet-Biedl syndrome (BBS). This will: (i) contribute to an advancement of knowledge, through open access publication in the international scientific arena, and (ii) enable translation of scientific advances into improvements in clinical practice. In general, short-term outputs will include the support to publish research articles and funding applications. Medium and long term outputs will be the development of new delivery techniques for gene therapy and understanding of mouse animals' models and our goal of this project, the cure or halting of blindness, obesity and associated diseases for Bardet-Biedl syndrome and other ciliopathies.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The overall aim of the project is to treat human patients with BBS and other similar disorders. BBS is a genetic condition that affects many organs, causing blindness, inducing obesity and cognitive impairment among many other tissues affected. BBS affect 1 in 100,000 new babies born in the United Kingdom. Children affected by BBS are born with normal vision but they go blind in their teens. So far, there is no treatment available to cure or halt the disease. We aim to find the best possible way to deliver the correct copy of the broken gene and we will use modified viruses to deliver it or replace the broken cells with new ones. Once we can demonstrate our system is working and it is safe in mice we aim to start clinical trials in human patients

How will you maximise the outputs of your work?

The outputs of this proposal will be the data demonstrating the phenotypical analysis of the mouse models and the efficacy of our treatments. Most of our main material will be in a digital format. Results will impact directly in the preparation of clinical trials, increasing their scientific value. Our sharing plan will consist in the dissemination of our results in seminars, conferences, peer review articles with open access. We plan to attend the British Society of Gene and Cell Therapy (BSGCT), European Society of Cell and Gene Therapy (ESGCT) and American Society of Gene and Cell Therapy (ASGCT) conferences and prepare talks and posters. We have a strong relationship with the BBS UK Society and other American and European BBS societies. They support our research and we annually disseminate our results at their conferences.

Species and numbers of animals expected to be used

- Mice: 12000

Predicted harms

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

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The project will use genetically modified animals that present the same defects that Bardet-Biedl syndrome patients and other related syndromes present. Blindness and obesity are not conditions that are hampering day-to-day living for the mice, as they have already a poor sight anyway, they are and they are not extremely harmful to the animals.

After birth we will inject our animals with our therapeutic products a maximum of two times. This injection can be intracranial, in the eye or intravenous. These are minor and quick procedures done under anaesthesia where the animals recover fast. Our protocols can last up 12 months after these injections. To follow the recovery of the treated animals we will monitor the function of the eye once every few months, weight every week and we will take small blood samples. Depending on the protocol we can also use imaging techniques to test once the brain function and structure.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Our procedures has been well stablished within the scientific community and are only considered of moderate harm for the animals. Therefore, our protocols are only considered of moderate harm to the animals. However, adverse effects could appear. In some cases we will need to anaesthetise the mice to perform protocols to deliver gene and cell therapies to eyes of brain. They could be infections, haemorrhage or unexpected secondary effects of injections and anaesthesia. On top of the injection procedures, there is a lot of information and plenty of data suggesting that regarding the vectors and cells we inject have no side effects and, proving they are very safe. We will design the experiments in advanced and we will know all the different tests we will want to check in treated animals after our treatment. After the test, we will kill the animals to further do more analysis and validate our approach to treat the disease. The final expected level of severity will be always moderate. We aim to be vigilant if unexpected severe adverse signs appear to euthanize the animals.

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 species)?

This project only will use mouse as model animal. In this project half of the animals are expected to have a mild severity and the other half of the animals a moderate severity procedures.

What will happen to the animals at the end of the study?

- 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?

Ciliopathies are inherited genetic diseases and are mostly multi-system disorders, and disease of one tissue or organ often has knock-on effects on disease in other organs or tissues. For example, in Bardet-Biedl Syndrome present brain pathology and in in the hypothalamus thatcauses massively enlarged body weight and hormonal misregulation.

We have demonstrated that correction of brain function has a direct impact in fat accumulation. Therefore, to identify the correct target for treatment we require a complete organism, a living animal,

and is something that cannot be achieved solely by in vitro or cell culture analysis.

Moreover, if you need to proof efficacy of your gene replacement product the only method accepted by all drug regulatory agencies (UK - Medicines and Healthcare products Regulatory Agency (MHRA), USA - Federal Drug Administration (FDA), EU - European Medicines Agency) is to demonstrate it in an in vivo whole animal model.

What was your strategy for searching for non-animal alternatives?

Nevertheless, all constructs are initially tested in cell culture using human cells lines. This is firstly to assess that the vector is functional and capable of expressing the transgenes in the manner it was designed for. When using vectors able to transfect in vitro cell lines (e.g. lentivirus), batch quality control analysis are performed to ensure that a gene therapy preparation is of a sufficiently high titre. These in vitro alternatives will always be used before any in vivo analysis.

Moreover, we are also continuing to investigate how human IPSC-derived cell cultures can be used to study gene therapy effects on different human cell types in vitro. Those studies have already developed three-dimensional organoids and esferoids such as eyecups and brain organoids that recapitulate some cell layers and structure of the tissues. However, those still cannot replace the metabolic interactions between different organs found in animals.

Why were they not suitable?

The alternatives described above will allow us to test the improved methods and materials in vitro. This way we will know which are the most promising constructs, cells or combinations of methods allowing us before we start the studies in our animal models.

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?

We have estimated the numbers of animals used based in our previous experience with this type of mouse lines. We also know the breeding requirements of our mouse lines. Our approach to the study and development in our research programmes allow us to calculate with precise accuracy what are the number of studies and the number of animals. For each programme we have tight timelines that ensure we won't over or underestimate the resources we need to compete them.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

-Careful experimental design. Where an outcome is quantitative (e.g. phenotype frequency), a power calculation determines sample size and professional statistical advice is sought where needed. Experiments are designed with reference to the ARRIVE Guidelines.

-For experiments with a qualitative outcome (e.g. pattern of gene expression in differing genotypes), 15-20 embryos will be examined per group to ensure reproducibility if the analysis in embryological for aim 1. For aim 2, neonates, we will be using between 10 and 14 animals per group. If the analysis is in adult animals 5-6 animals will be used per group. All this number will be determined by the primary end-point for each experimental protocol, according to power calculations described below.

For the testing of viral vectors for efficacy, we will prepare a careful design studies to reduce the number of animals without losing the statistical relevance of the readouts.

-We will prepare multifactorial experiments where multiple quantitative outcomes will be tested at the same time to avoid repeating experiments. Our experience indicates that the weight is the variable that displays more internal variability within the mutant animals compared with outer nuclear layer depth, retinal ERG function or central nervous system ventricle volume. Therefore, our sample size calculations will be based with this variable, using the difference in weight observed between wild-type and obese mutant animals. Design and calculations will be following ARRIVE guidelines and performed by the Power and Sample Size Home (http://PowerAndSampleSize.com) tools, Sample Size Needed to Compare 2 Means:2-Sample, 2-Sided Equality, recommended by the N3CRs experimental design (https://www.nc3rs.org.uk/experimental-designstatistics).

What other measures apart from good experimental design will you use to minimise numbers?

1. Efficient breeding. Our experimental procedures are based on time mated breeding and our experimental designs are telling us the numbers of animals needed for each procedure. Therefore, we will only use the exact number of litters for each protocol.

2. In order to test the efficacy of our treatments we need to know the exact variability of the readouts to prepare the experimental designs. When describing the phenotype of a new transgenic mouse line we will measure in detail each variable with pilot studies to obtain the best data.

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.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Mice will be the animals used in this project. To investigate human genetic conditions mice are the best and only model. Mice are mammals, they share most of the genes with humans, when we mutate a gene in mice it usually shows the same symptoms as the patients. We even have models that have the exact same mutation changed that is found in some of our patients, and they have the same defects we find in humans.

Another reason to use mice is that the therapies we are testing work in a very similar way in mice and humans. This allow us to be certain that the results we observe in the lab can be reproduced in humans. The injections we give to the animals in the eye and the brain are not complex surgeries. However, we will be careful monitoring the animals during and after the injections a few times a day as the most probable adverse effect could be infection after the procedure. With the help of the veterinary we will decide if we need to use painkillers or antibiotics if we find animals in this situation. The therapies we give are not expected to have any secondary effect in the animals either, but we will also monitor the animals carefully to see is there are signs of distress or change in their behavior due to our therapies. This information will be also very relevant to understand if our therapies are safe to use in humans.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Mouse models of ciliopathies recapitulate very faithfully the cellular and molecular In order to test preclinically our therapies the regulatory bodies.

What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

The delivery routes we propose has already been extensively tested delivery routes proposed here are the most refined delivery routes that can be used to obtain thenecessary information. Strict monitoring of animal health after the proposed delivery routes is the most refined way to produce the desired data.

Surgery will be carried out with aseptic technique to minimise the risk of infection. Animals may suffer mild discomfort following the procedure once they recover from general anaesthesia i.e. piloerection, reduced movement, ‘nesting’. This will be alleviated using systemic pain relief in consultation with the NVS (meloxicam or buprenorphine).

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow the updates on the ARRIVE guidelines published (https://arriveguidelines.org/). For surgery we will follow the LASA Guideline or Preparing for and Undertaking Aseptic Surgery (https://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf)and follow their news, updates and recommendations (https://www.lasa.co.uk/info/). We will also refer to the PREPARE guidelines: (http://journals.sagepub.com/doi/full/10.1177/0023677217724823]) and its updates.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Our lab is in close contact with the N3CRs, submitting grant applications to improve the therapeutic methods, with submissions to CRACK IT Challenges (https://nc3rs.org.uk/crackit/crack-it-challenges). We are aware of the documentation published and provided by the N3CRs (https://nc3rs.org.uk) and attend their webminars and presentations.

Explain the choice of species and the related life stages

The mouse models of ciliopathies we will be using are the smaller mammal that show exactly the symptoms and organ degeneration found in human patients with the same disorders. The progression of the disease like retinal degeneration and brain problems, is also very similar to the one that patients suffer. For this reason, they are the best type of animal we can use to study them.

Because of the disease progression is similar between mice and humans, we need to choose the same stages that are important for patients also in the mice. For that reason, we will study and treat the animals with our therapies at the same stages that offer a window of opportunity to treat our patients.