Home Office

NON-TECHNICAL SUMMARY

Modelling gene therapy for congenital bladder dysfunction

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
- (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

therapy, congenital, urinary, genetic, neuro-muscular

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant, 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 is to take the first experimental steps to cure congenital diseases that affect the nerves and muscle of the bladder and prevent normal urination. We will gain biological insights into these diseases by studying genetic mouse models. We will use these models and the biological insights as a platform to test whether gene therapy can safely cure these diseases.

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?

Nearly 30 in 10,000 live births have congenital anomalies of the urinary tract, the 3rd most prevalent anomaly in the UK, and one of the commonest disorders detected when prenatal ultrasound screening is performed. Some severely affected foetuses undergo termination while others are born. Of those born, many suffer from kidney failure as children or adults, and require dialysis or transplantation, without any cure for the underlying pathology. One particular subset of anomaly affects nerve – muscle communication and as such causes functional, rather than anatomical, obstruction of the urinary tract.

In the last 25 years, genetic causes of many congenital diseases have been defined, opening the possibility of developing cures that target the underlying molecular pathology. Effective cures for diseases with a genetic basis are being sought in other disease systems, utilising understanding of the genetic and biological disease pathology, for example in Spinal Muscular Atrophy. Here, modified viruses are used to deliver therapeutic genes to the affected tissues. This has not been attempted for diseases that affect the urinary tract, despite the associated health burden and an increasing understanding of the genetic bases for these diseases. Neuro-muscular diseases that affect bladder voiding are a promising target for gene therapy as there is no anatomical anomaly per se, opening the possibility that by resolving the underlying defect at a gene level we will be able cure the disease. To establish whether gene therapy could be efficacious it is essential to fully understand the disease processes, and how these change with age (the disease natural history). We will use this knowledge to design and test therapeutic strategies in animals that have the disease. This will pave the way for evaluating the safety and efficacy of therapies in people with congenital voiding dysfunction.

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

This work will demonstrate the feasibility of using gene therapy to treat mouse models of bladder voiding dysfunction. Such studies have not been performed previously in the urinary tract and the results will form the basis of multiple high impact publications. Totally novel information will be obtained on the feasibility of using viral vectors to deliver therapeutic molecules, and treatment parameters will

be defined. Crucially, the efficacy of using viral vector-delivered gene therapy to prevent congenital bladder dysfunction will be determined. If the pre-clinical trials described in this licence are successful, they will pave the way for use in human clinical trials.

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

There is no cure for congenital bladder dysfunction, and affected individuals are at a high risk of renal failure and death, with costly continual treatment. Therefore, there is an urgent need to develop new treatments. In the short term, these studies will set a precedent for how advanced therapies, such as using viruses to deliver gene therapy, can be used to treat animal models of voiding dysfunction. This will be of great interest to various parties, including urologists, researchers that study the urinary tract, and researchers who are involved in the application of advanced molecular therapeutics.

From a patient perspective, these studies are designed to lead to clinical trials and as such will be of potential direct benefit to affected individuals. Here, we can look to the precedent set in the treatment of spinal muscular atrophy, where successful preclinical trials of viral vector mediated gene therapy were run in genetic mouse models of the disease. This subsequently led to human trials and regulatory approval in USA and EU in under eight years, with treated children effectively cured.

From the perspective of policy makers and funding bodies, this work will demonstrate another disease system in which gene therapy can be efficacious. We are working closely with large program bids to attract funding for Rare Disease research, and this work will contribute to the relevance of ‘personalised medicine’ in treating rare and costly diseases.

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

Our results will be published in original research papers in high impact journals. We will report ineffective therapeutic strategies as well as the effective strategies, an essential step in the development of novel therapeutics. We will also discuss our work at international conferences, such as the International Continence Society, and we have collaborations with both gene therapy and urology experts.

Species and numbers of animals expected to be used

- Mice: 4000

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.

Urinary voiding is a complex, multi system behaviour. Mice model many of the physiological aspects of human voiding, and there are genetic mouse models for diseases that affect bladder voiding, including

a number with a neuro-muscular basis. It not possible to model the full range of physiological defects and therapeutic effects in non-mammalian systems. We will study embryonic and postnatal to adult mice, to identify the developmental origin and natural history of the diseases being studied. This will inform our therapeutic strategy by indicating a therapeutic window. In addition, we also require adult mice to maintain our genetic mouse colonies.

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

Mice will be bred from parents that each have one copy of a mutant gene, resulting in ¼ offspring with both copies of the mutant gene. These mice will have bladder voiding dysfunction. Within 48 hours of birth, the mouse will receive an injection of the virus containing the therapeutic gene, into the superficial temporal vein. The therapeutic administration causes no lasting harm to the mouse, but can involve anaesthesia. The mouse will be observed and analysed by non-invasive techniques, such as ultrasound or voiding behaviour evaluation for up to eight weeks. The mouse will be humanely killed or will undergo a physiological technique to measure bladder function, while under terminal anaesthesia, and then be humanely killed. Some mice that are not used to model a therapy will be studied for up to one year to understand the underlying disease mechanisms. While alive, they will be studied with the same non-invasive techniques and then humanely killed or analysed for bladder function while under terminal anaesthesia.

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

Mice will be bred from parents that each have one copy of a mutant gene, resulting in ¼ offspring with both copies of the mutant gene. These pups will have bladder dysfunction and may develop mild urinary tract malformations, which is compatible with normal health. There is a small possibility of significant dysfunction developing, which would put mice at risk of developing kidney failure. While this condition is not painful it does cause ill health. Accordingly, mice will be monitored from birth and those showing persistent signs of ill health will be humanely killed, to ensure the project does not exceed moderate severity. Homozygous mutants in our established Hpse2 and Lrig2 mutant lines show slower weight gain compared to their littermate controls. We will monitor litters for signs of distress and weight gain, and cull any mice that show signs of distress or abnormal behaviour, or if weight gain is sufficiently slow that they approach 50 % weight of littermate controls.

Pups up to two days old will be treated by injection of therapeutic molecules into the superficial temporal vein in the head. This route of delivery does not cause any lasting harm to the mouse, but there is a risk of maternal rejection when the pup is returned to its mother, and there is risk associated with anaesthesia, if used. To minimise this risk we will allow the mouse to ‘pinken’ on a warmed pad before return.

The viral vectors we use have been extensively studied in animal models and have been approved by the EU and the US FDA for clinical use. They are non-pathogenic, are engineered to be replication and integration deficient, and the risk of an auto-immune response is exceedingly small in laboratory animals. The therapeutic molecules that will be delivered by the viral vectors are widely expressed in wildtype mice and each transgene will be individually assessed for risk in the relevant GMO form.

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)?

The heterozygous with wildtype breeding animals are all expected to have mild severity; offspring of heterozygous with heterozygous parents may experience moderate severity depending on their genotype. Treated mice will also experience a moderate severity. Overall, 2/3 of animals will experience a mild and 1/3 a moderate severity.

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

- Killed

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?

Voiding is a complex, multi system behaviour. Although there is considerable data on gene expression patterns in the organs of the urinary tract these are not sufficient to allow an understanding of the anatomical and physiological perturbations that are caused by mutations in these genes and it is not possible to model the full range of physiological defects and therapeutic effects in non-mammalian systems. Given the need for realistic preclinical models, there is currently no alternative to using live animals. Rodent urinary voiding models many of the physiological aspects of human voiding, and there are faithful genetic mouse models for many diseases.

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

We have used cell lines, including nerve cells and muscle cells, to model specific aspects of the genetic diseases and test the therapies described in this project. For example, we test the ability of each viral vector to get into the cell and drive expression of the therapeutic gene before we use the vector in the animal model. Viruses are applied to cells in culture and the amount of gene being produced will be assessed quantitatively, and visualised to see if it is in the expected part of the cell.

Why were they not suitable?

Cell lines and other in vitro techniques do not model the complex interplay between multiple tissue types that is necessary to understand these genetic diseases. Delivery of therapeutics to cell lines gives some indication of the potential efficacy of the strategy but do not offer further insights into how these technologies will behave in the complex environment of a living animal. For example, it is also not possible to model an immune response in vitro or how other organs will be affected. It is essential to gain knowledge on all of these aspects of the disease and the therapeutics as the fundamental aim of the project is to bring these technologies into the clinic to treat patients.

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?

Extensive experience with animal models in previous similar projects indicates that 2000 animals will be required for five years to maintain the multiple colonies used in this project, while a similar number will be required for the specific experimental protocols.

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

We will use non-invasive techniques to obtain a large amount of data for each mouse. We will use scans to monitor the structure of the renal tract, which require anaesthesia but are minimally invasive and can be used recurrently on a single mouse to monitor disease. We will analyse voiding behaviour with the voiding stain on paper technique, which simply involves placing a mouse on blotting paper for some hours then imaging the urine stains, but gives a lot of information on the ability of the mouse to void.

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

Each experiment has been assessed by to determine the optimal number of mice. We have considerable previous experience and data from animal models to do this accurately. Our group also has great experience in efficiently running mouse colonies to ensure the minimal required numbers of mice are bred.

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.

The models we will use are faithful genetic models of human voiding disease, and as such will themselves have urinary voiding dysfunction. The primary defects do not cause significant distress to the animals and we will humanely kill any animal that displays signs of secondary disease, such as

kidney disease. We use non-invasive techniques multiple times on individual animals to maximise the data gathered from a single animal, without causing harm. We have refined our techniques in line with changing good practice and our own expertise, for example in regards to the superficial temporal vein injection into neonates. Originally, we used both anaesthesia and ice-induced hypothermia. Along with our colleagues, we have determined that the ice is not necessary to immobilise the mice and we have removed this step from the protocol. We will receive training to develop our pup restraint technique to not require anaesthesia, as this bears some risk to the pup. In addition, as our understanding of the therapeutic vector efficacy develops, we will further refine this protocol by selecting only the mutant mice and appropriate controls for treatment - this will require tattooing pup foot pads, taking tail clips from the neonatal mice and rapidly genotyping the whole litter.

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

To accurately model the efficacy of therapies it is essential to use animals with mature voiding function that is similar to adult human voiding. We have intensively studied *Hpse2* and *Lrig2* mutant mice and confirmed they have bladder voiding dysfunction similar to that seen in human patients with urofacial syndrome (other groups have studied mice with mutations in *Chrm3* and *ChrnA3* and also identified bladder dysfunction). In addition, there are no non-mammalian species that provide a sufficiently faithful model of normal human voiding, or of human congenital bladder disease.

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

We will stay informed of updated and variant protocols being used in the scientific community, such as best practice for temporal vein injections, by reading the literature and discussing at meetings.

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

We will follow LASA guidelines and NC3R guidance.

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

We will regularly check the NC3R website for changes in best practice advice, and engage with the animal facility for guidance on other advances.