Home Office

NON-TECHNICAL SUMMARY

Development of a new generation of AAV vectors to treat monogenetic disorders and acquired conditions

Project duration

Years **5**
Months **0**

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

AAV, Gene therapy

Animal types	Life stages
Mice	adult, aged, juvenile

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 is the aim of this project?

Our research programmes focus on the development of potentially curative treatments for several monogenetic disorders and rare acquired diseases, with a largely unmet therapeutic need, using advances in gene therapy technology. Based on the great success of haemophilia B program, as the main aim within the next 5 years, we will extend its focus to different inherited and immune-mediated disorders, using new vector design that enables therapeutic expression of transgenic protein in appropriate mouse models without toxicity.

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?

We have used our gene therapy approach for treatment of patients with haemophilia B, an inherited bleeding disorder, who are currently participating in our Phase 1-2 clinical trial. So far, we have observed that endogenous 24/7 expression of the missing clotting factor (factor IX) prevented micro-bleeds into joints. This will reduce the subsequent need for expensive and life-threatening joint replacement surgery, thus adding to the additional economic and social benefits. During this trial we have learnt that the efficacy of this specific gene therapy approach in mice predicted success in humans, indicating that evaluation of any new vector in a mouse model is essential. We have also learnt that immune response to gene therapy (pre-existing and/or acquired) can hinder the efficacy of gene therapy and prevent re-dosing. Understanding the mechanisms underlying this immune response is of crucial importance for future success of the AAV-focused gene therapy.

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

We expect to demonstrate that gene therapy with AAV vectors will be safe and effective in the treatment of a range of monogenetic disorders, especially those that remain a significant burden on the healthcare and society. The new vectors being constructed for these disorders are likely to be more potent than has been the case to date. In addition, the quality of vector is improving over time. These advances reduce the risk of toxicity whilst reducing the amount of vector required for the effective gene transfer. The results we obtain with our new vectors will provide new information about the new therapeutic approaches for the explored diseases and will be published in scientific publications to disseminate the knowledge acquire to a wider scientific/clinical community.

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)?

Our work has recently established the first global proof-of-concept for a gene therapy approach to the treatment of severe haemophilia B. This entailed a single intravenous administration of an AAV encoding an optimised FIX gene, resulting in a long term (>6 years) dose dependent increase in plasma FIX levels at therapeutic levels without persistent or late toxicity. Gene therapy, therefore, has transformed the lives of patients with haemophilia B who participated in our gene transfer study and has resulted in a saving of >£5M to the NHS just from the reduction in the use of factor concentrates. Successful gene therapy can also have benefits for the whole society via reduction in absenteeism from work or school and reduction in demand on social services. In addition, the work proposed will help us develop novel gene therapy approaches for other disorders where current treatments are not optimal.

How will you maximise the outputs of your work?

In our pursuit of the most appropriate methods for testing our vectors, we will form many international collaborations with key opinion leaders in the field, both academic and industrial to join efforts in our attempts to find the best AAV approach for various indications with not just therapeutic but curative potential. We will make sure our results, both positive and negative, will be published in peer-reviewed scientific publications and made available for the wider community.

Species and numbers of animals expected to be used

- Mice: 4500

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.

In a typical experiment, animals will be allowed a period of acclimatisation before any procedure is performed to reduce stress. Biological samples are collected before starting any treatments to have a benchmark data. The animals are then injected with a gene therapy vector and the biological samples are collected at regular intervals to monitor the effects of the gene therapy. The minimum duration of an experiment can be 4 weeks or up to 24 months in long-term experiments. At the end of the experiment, the animals are humanely killed and several samples and biological fluids are collected for extensive analysis. In the experiments focused on specific disease models, animals will be allowed a period of acclimatisation before any procedure is performed to reduce stress. Biological samples are collected before starting any treatments to have a benchmark data. The animals are then injected with a gene therapy vector and allowed a period of up to 4 weeks before the pre-clinical models of diseases are started. For example, changing the standard rodent diet to a diet that causes the animals to accumulate fat in the liver (a model of non-alcoholic steatohepatitis), or injecting or applying chemicals that cause local or systemic inflammation and tissue scarring (similar to a range of auto-immune diseases). During the progression of the disease models, biological samples may be collected at regular intervals to

monitor the disease progress. At the end of each respective pre-clinical model of disease, the animals are humanely killed and tissue samples, in addition to biological fluids are collected for extensive analysis.

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.

Most of our protocols and procedures are not expected to cause adverse effects that are long-lasting. However, animals can experience discomfort and pain from the injections and/or sample collections. We will monitor these and provide pain-relief as needed. Other effects of treatments that we will monitor include changes in body weight and behaviour and general overall condition. We anticipate that adverse events will be transient (1-6 hours) and any animal showing adverse events that do not improve within will be humanely killed. In the experiments focused on specific disease models, we have proposed the most refined disease models that result in similar clinical outcomes that are observed in humans. Most of the disease models are expected to cause mild-moderate adverse effects as the disease models progress. This can typically cause modest weight loss which will be an important indicator of the welfare of the animal. We will monitor the animals frequently and provide appetising gel diet to counter the weight loss. If this does not cause an improvement, animals will be humanely killed. We have established clear endpoints in all models that do not cause unnecessary discomfort and pain to the animals, yet will allow us to address our scientific questions.

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)?

The degree of severity is expected to be moderate or less depending on the procedures we propose to use. All of our protocols will be performed on mice. Mice will be closely monitored for development of any adverse clinical signs and clear endpoints are defined in the protocols in order to minimise conditions of distress. At the end of each experiment and at well specified humane endpoints, mice will be humanely killed and blood and tissues will be collected for analyses.

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

- Killed

Application of the three Rs

1. 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 have previously demonstrated the therapeutic potential of gene therapy in a first-in-human clinical trial for the treatment of inherited bleeding disorders that are caused by genetic mutations in blood clotting factors. The treatment options for bleeding disorders do not cure the disease, rather, they control the symptoms or severity. Moreover, these treatment options involve multiple hospital visits, are very costly and have a burden on the patient and the NHS. Our gene therapy trial has already saved the NHS over £5 million and can be considered a potential cure as for more than 6 years, the endogenous levels of the blood clotting factors are stable following the single injection of our gene therapy. Therefore, we are now aiming to use our unrivalled technical expertise and experience in the gene therapy field to further improve how our gene therapy vectors work to treat different genetic disorders and acquired, chronic disorders that have a significant socio-economic burden.

All of our proposed work using animals will be preceded by studies using mouse/human cell lines including stem cells, and organoids (miniature organ-like 3D cluster of cells), and organ-on-chip cellular assays which are becoming increasingly important in modelling different disease conditions. These crucial studies will allow us to select the appropriate gene therapy vectors for evaluation in mice. The core physiological systems of the body including the immune system, are controlled by complicated inter-dependent mechanisms that are not replicated by cells grown in a laboratory. The mouse represents the lowest vertebrate species that is accessible to genetic manipulation for the understanding of mechanisms of diseases. Additionally, mouse and human genes are similar by approximately 80%, which would allow us to predict the therapeutic effects of our gene therapy vectors in human patients. There is a large amount of published scientific body of work that shows that our proposed models have a good degree of translation to the effects observed in humans. Importantly, all of the procedures that will be undertaken have been refined to minimise suffering.

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

1. Cell culture-based approaches - Where possible the gene therapy vectors we develop are assessed during in vitro assays using commercially available mouse and human cell lines to ensure efficacy. This is employed for all our approaches and for each new batch of vector.

2. We are developing and evaluating disease specific human cell models obtained from stem cells to determine if these can replace animal models for evaluation of gene therapy vectors.

3. We are evaluating human liver cells grown in 3D cultures (organoids) to see if these can substitute mice for assessment of gene therapy vectors.

4. We are currently evaluating ‘organ-on-chips’ assays that use human cells to model a physiological system (e.g., the liver) and which can be manipulated to mimic clinical conditions (e.g., fatty liver disease). This will allow us to assess the efficacy of our candidate vectors in the therapeutic indication before commencing animal studies.

Why were they not suitable?

Murine models of human disease serve as an important tool for establishing pre-clinical proof-of-concepts, and for assessing the efficacy and safety profile and immunological consequences of our gene therapy approaches. Based on the clinical data becoming available from clinical trials, it is becoming increasingly important to understand the effect of the host immune response on the efficiency

of gene transfer after gene therapy treatment. The body's response to gene therapy vectors involves multiple systems, organs and cell types. Additionally, the complexity of the immune response cannot be assessed in in-vitro settings. The use of in vivo models is essential to refine existing therapies, and develop new therapies, as they will allow us to detect the persistence of the gene therapy, their efficacy in modifying/preventing diseases, and any potential toxicity. In vitro systems, while useful, do not fully replicate the complexity of immune interactions or disease mechanisms in vivo and it is essential to use appropriate and robust animal models to understand these processes.

2. 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?

The estimation of the numbers of animals intended to be used under this PPL has been agreed with project leads at our company and aligned with our strategic goals for the next 5 years. Additionally, we have also used our experience from our previous project licence to estimate the number of animals that we will use.

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

We have used Power calculations using the NC3R's Experimental Design Assistant to reduce the number of animals being used whilst ensuring that sufficient data is obtained to answer the research question. Additionally, we have designed longitudinal pharmacokinetic/pharmacodynamic experiments that will allow us to obtain data from the same animal at different time-points, instead of using different animals for the distinct time-points. These experiments will be performed at the highest standard that will not compromise the welfare of the animals and will also allow us to significantly reduce the numbers of animals being used in this project. Similarly, we designed experiments that will allow us to maximise the information obtained from each animal via advanced molecular biological assays on post-mortem tissue/biological samples following the completion of in vivo experiments.

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

In addition to good experimental design, we will extensively use computer modelling and cell-based evaluations to reduce the number of animals used in our project. We will also assess sharing tissue samples from different experiments to address specific objectives, if feasible. We will consult with the experienced animal care support staff at our animal facility to implement strategies that will allow us to maintain efficient breeding of animals. This may involve freezing of sperm and embryos to reduce the number of animals kept alive for specific disease models. Importantly, our study designs will be reviewed by statisticians to ensure that we design experiments that will use the minimum number of animals to achieve our objectives.

3. 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?

The majority of our animal models are genetic or induced to resemble human disease conditions. For example, we will use mice that are genetically modified to model inherited human conditions (for example inherited bleeding disorders). These mice do not have the severe phenotype seen in human patients but have the same dysfunction (i.e. in their blood clotting system), which allowed us to use this model to successfully treat human patients. We are now proposing the use of different pre-clinical models of chronic diseases using models that are known as gold standard. These models closely resemble the human patients with the least suffering and distress caused to the animal. We will design experiments that will allow us to determine the efficacy of our candidate gene therapy vectors in improving these conditions by injecting the gene therapy vector by different routes. The number and volumes of administrations and blood samples will be minimised while ensuring scientific validity and minimising discomfort. Before any experiment is performed, animals will be acclimatised to procedures. Environmental enrichment will be provided in home cages.

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

We have chosen to work with mice as they are the lowest vertebrate group with well characterised disease models. The mouse models we propose to use in this project license are already well established, characterised and described in the scientific literature.

As described before, unfortunately, in vitro studies do not completely predict outcomes in humans. For example, a common gene therapy vector (AAV8) performed poorly in vitro but were highly efficient in animal models including mice. Efficacy in mice predicted the outcomes in human patients with inherited bleeding disorders (haemophilia B). Importantly, the mouse immune system is the best characterised amongst vertebrates and there is a strong degree of similarity between human and mouse immune systems. Most reagents and tools required for the proposed plan of work have been designed for use in mouse models and for the detection and tracking of murine immune cells. Immunological assessments are going to be an important part of the studies we will perform over the next five years.

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?

All the protocols have well defined end points and mice will be humanely killed with an appropriate Schedule 1 or non-schedule 1 methods within 12 months of receiving gene therapy vector or earlier where indicated. In some studies, an extended duration may be required (16-24 months). Full effort will be made to ensure animal well-being and comfort, and to minimise pain and distress. Good handling will minimise discomfort of the animal during the procedure. Sites of injection will be monitored carefully

to prevent wound infection. If needed, we will apply local analgesics over the affected region to improve recovery. Aseptic techniques will be used at all times. Experimental animals will be monitored at least daily by our research group and staff within the animal unit. Details of animal experiments in progress will be shared between our research team and the staff within the animal unit to ensure that unexpected adverse events are quickly resolved to minimise harms to the animal.

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

We will use the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines in addition to Home Office published guidelines (e.g., Guidance on the Operation of the Animals (Scientific Procedures) Act 1986) and relevant LASA guidelines. Additionally, all murine models that we propose to use have extensive literature resources available which will allow us to conduct our experiments in the most refined way.

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

We currently receive news bulletins and invitations to specialist seminars organised by the NC3Rs and our animal facility is very engaged with the NC3Rs community. We will maintain this engagement to stay informed about the advances in the 3Rs, in addition to staying up-to-date with scientific publications that highlight 3Rs principles. We will implement these advances effectively by working with the animal facility technical staff and our research team.

Explain the choice of species and the related life stages

Mice are the lowest vertebrate with well characterised disease models including genetic manipulation that is used to model human inherited diseases. No other species could fulfil the requirement of this programme, especially in term of generation of pre-clinical proof of concepts, to the same extent as the mouse. Most of our proposed protocols will use animals at the adult stage to ensure that all the physiological systems are functionally and fully developed. This is also relevant in terms of refinement, as we will avoid using juvenile or aged mice, without a valid scientific objective.