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NON-TECHNICAL SUMMARY

Pathology and Treatment of Lysosomal and Related Diseases and Brain Tumours

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

Therapy, Inflammation, Lysosomal Disease, Brain Tumour, Neurological Disease

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged
Rats	neonate, embryo, juvenile, adult, pregnant, aged
Sheep	adult, 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 projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this project is to develop new treatments for lysosomal and related diseases (LSDs), such as Mucopolysaccharidoses (MPS) and brain tumours through better understanding of how they are caused. We want to study how inflammation changes the course of disease and will develop new therapies using this knowledge and test the quality, effectiveness and safety profile of them in both mice and sheep prior to clinical trials.

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?

Lysosomal and related diseases are inherited genetic disorders which lack specific enzymes that breakdown complex sugars, fats or proteins in a compartment of the cell called the lysosome. These include progressive childhood metabolic lysosomal diseases such as mucopolysaccharidosis (MPS) types I, II, IIIA, IIIB, IIIC, IV, VI and VII, Krabbe disease, Wolman disease and many others. A build up of undegraded by products results in often severe inflammation, blocks to some signalling pathways, damage to organs and in some cases severe damage to the brain resulting in behavioural difficulties and death before 20 years of age. Some of these diseases such as MPSIIIA, and B primarily affect the brain making treatment development very challenging. Brain tumours typically include the most common glioblastoma, which invades the brain and also causes severe inflammation. All of these diseases primarily affect children or young adults and most have very poor existing therapies. As they are individually rare, it is important to find commonalities between the diseases to aid in the development of future therapies. Current treatments are unable to correct the brain damage seen in many forms of lysosomal diseases, especially MPSIIIA and MPSIIIB since enzymes are unable to enter the brain. Bone marrow transplant is curative for a small subset of lysosomal diseases, but not all, and has even been tried for brain tumours with mixed results. Aside from supportive care, there are no effective treatments for these severe diseases and therefore they represent an urgent unmet clinical need especially with regard to treating the brain. Furthermore, each of these diseases provide a huge cost to the NHS, with enzyme treatment for an average child with a lysosomal disease at £250,000 per year. There is a large gap in our knowledge of pathology in these conditions and where disease treatment thresholds lie.

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

Short-term benefits: Improved information on inflammation in brain tumours and lysosomal diseases, understanding of the role of IL1 in neuroinflammation and behaviour, outcome of phase I/II trial of Stem cell gene therapy in MPSIIIA.

Medium Term: Further development of stem cell gene therapy clinical trials for MPSIIIA and initiation of clinical trials for MPSII and MPSIIIB. Refinement of brain delivery of haematopoietic stem cell gene therapy, improvement of direct brain gene therapy delivery methods in sheep.

Long term: Clinical trial of direct brain gene therapy for MPSIIIC using methodology developed in sheep, development of anti-inflammatory therapies in MPS diseases and expanding their use to other orphan diseases, development of new cell therapies for lysosomal disease, understanding of respective functional roles of inflammatory cells in tumours, development of novel therapies for brain tumours, clinical market authorisation of stem cell gene therapy for MPSIIIA.

We expect to publish our work in scientific journals and at conferences and to be able to generate new patents around methods of treatment -ideally for several diseases at once, and either commercial licences or a spin out company to take these advances forward into patient therapies.

In addition, we will build on our existing tissue bank of samples for collaboration. Our repository of scanned slides provides an avenue for furthering collaborative understanding of inflammatory and disease processes across multiple diseases.

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

There are currently no adequate treatments for the conditions we work on, as such the main benefits of this project will be the development of novel treatments that could make a significant impact in the lives of these children. The work will improve how we use bone marrow transplant currently and in combination with other treatment strategies which are most appropriate for therapy in the brain and help to bring them to clinical trial more rapidly.

These data will be of paramount importance for the development of 3 novel therapies for lysosomal diseases and brain tumours in the next 5-10 years and in the short-term will inform stem cell biologists, transplant surgeons and immunologists both nationally and globally in the interim with our research findings on stem cell engraftment. The National Institute for Clinical Excellence has begun to reject enzyme replacement therapies, beginning with the Morquio drug Vimizin (later reversed), due to the poor cost benefit relationship of these products (£250K/annum/patient). These costs are not sustainable, thus the gene and drug therapies that we develop are key to solving this crisis.

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

We will maximise outputs through several means. We will collaborate with groups both within the establishment and elsewhere to generate and share knowledge of this diseases and models. We will look to publish all findings, even when unsuccessful. We will disseminate knowledge through conference presentations and publications.

Species and numbers of animals expected to be used

- Mice: 15,000
- Rats: 150
- Sheep: 60

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 are the least sentient species in which models for lysosomal diseases exist and provide some of the best available models of human disease progression. Mice are typically used between 2 months of age and 12 months of age. Many of the diseases we work with are childhood diseases and therefore working with young mice more accurately represents the human disease. Occasionally we need to use rat models of disease if the mouse models don't adequately replicate disease characteristics. Brain tumour models, especially patient derived tumours implanted into adolescent immunodeficient mice, or genetic models from birth in mice provide some of the most translatable models to human cancers.

Scaling therapies up from the mouse brain to humans is difficult, therefore the use of a large animal model is required to optimise delivery routes and doses for later human trials. Many people are focussed on concentration of gene therapy products and volumes, whilst in confined and easily damaged spaces like the brain, flow rate and achieved pressure may be more important. Sheep provide a good representative model of humans in terms of brain anatomy and structure, blood supply, metabolism and lysosomal storage. Even the density and spacing of neurons in sheep brains better models a human brain than mouse brains. A sheep's brain at 140g, is the same size as a large monkey and is closest in size to children for any available large animal model.

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

Most animal models are bred from birth with a genetic disease, and many of these are not initially harmful to animals. Over a period of several months models of lysosomal and related disease and immune models start to show behavioural changes, reductions in joint mobility and evidence of inflammation, depending on the disease and most have a shortened lifespan. We usually implant patient derived brain tumours into immuno-deficient mice to make brain tumour models for subsequent treatment and some models are very slow, so we use drugs to make them more rapid to more closely model human disease. Other brain tumour models are initially harmless until the injection into the brain of an activating virus. We closely monitor harmful effects on animals and introduce mitigating measures where appropriate or cull animals where required.

Typical protocols for rodents include:

1) phenotyping to understand disease. Typically 2-3 times for each of the following over the several months lifetime of an animal:, an animal may undergo non-invasive behavioural tests, generally involving brief (10-30 mins) tests with no pain suffering and distress or food withdrawal for more than 16 hours. Intervals between tests will be at least 10 minutes and the maximum number of individual

tests or trials will be five in any 24 hour period, with no more than 24 tests or trials in a month. Animals may have retinal imaging under anesthesia. During the follow-up period, samples of bodily fluids (blood, saliva, urine) may be taken to analyse the effects of treatments and/or the health of the animal on up to 10 occasions as well as non-invasive imaging interventions on no more than 4 occasions which typically require anaesthesia and sometimes injection of a radiotracer (2-4 hours), contrast agent or light emitting compound. Animals may be culled under general anaesthesia.

2) bone marrow transplant: In addition to phenotyping tests above: A 2-4 month old animal may receive 2 injections to mount an immune response, then receive injections of chemotherapy over 1-5 days to kill off existing stem cells, with a day's break and then receive transplant of gene modified stem cells by injection to replace those killed with chemotherapy. They may then receive further injections of substances to determine if their immune system recognises the new gene as their own. All are extremely brief. Usually all the phenotyping happens after these steps.

3) delivery of multiple therapies: In addition to phenotyping tests above: A 2-4 month old animal may receive up to four therapeutic substances by multiple routes via up to 12 injections, where no more than 2 injections can be given in 24 hours, in combination with bone marrow transplant, in combination with a non-invasive therapy e.g: An animal receives therapeutic drug in drinking water (non invasive), then up to 4 injections of enzyme replacement (each less than 5 mins), then receives injections of chemotherapy over 1-5 days to kill off existing stem cells, with a day's break and then receive transplant of gene modified stem cells by injection to replace those killed with chemotherapy (each less than 5 mins) and then after at least 3 weeks recovery receive 6 injections into the brain of a therapy under anaesthesia (Typically 30 mins to 2 hours). They may then receive further injections of therapeutic substances, such as enzymes. A total of 18 injections in the worst case scenario. Usually all the phenotyping happens after these steps.

4) Immune priming and transplant: In addition to phenotyping tests above: A 2-4 month old animal will receive several injections to mount an immune response, this is expected to worsen disease. All are extremely brief over a few weeks, then may receive injections of chemotherapy over 1-5 days to kill off existing stem cells, with a day's break and then receive transplant of gene modified stem cells by injection to replace those killed with chemotherapy. They may then receive further injections of substances to determine if their immune system recognises the new gene as their own. All are extremely brief. Usually all the phenotyping happens after these steps.

5) Brain tumour implantation and treatment:In addition to phenotyping tests above: An animal will have a tumour implanted in the brain by injection or a virus to activate tumour growth. An animal may receive up to four therapeutic substances by multiple routes via up to 12 injections, where no more than 2 injections can be given in 24 hours, in combination with bone marrow transplant, in combination with a non-invasive therapy e.g: An animal receives therapeutic drug in drinking water (non invasive), then up to 4 injections of therapeutic drug (each less than 5 mins), then receive injections of chemotherapy over 1-5 days to kill off existing stem cells, with a day's break and then receive transplant of gene modified stem cells by injection to replace those killed with chemotherapy (each less than 5 mins) and then after at least 3 weeks recovery receive usually 1-2 injections into the brain of a therapy under anaesthesia (Typically 30 mins to 2 hours). A total of 18 injections in the worst case scenario. Usually all the phenotyping happens after these steps.

Typical protocols for sheep include:

6) delivery of therapeutic substances: Sheep models may either be bred from birth with a genetic disease, and many of these are not initially harmful to animals or we use normal sheep. A 2-4 month old animal may receive therapeutic substances by multiple routes, but mainlyvia up to 10 injections into the brain, e.g: An animal receives 6 injections into the brain of a therapy under anaesthesia, with one supplementary intra spinal and two cerebrospinal fluid delivery (brain spaces) injections (Typically 3-7 hours). During the follow-up period, samples of bodily fluids (blood, saliva, CSF) may be taken every 2 months or so to analyse the effects of treatments and/or the health of the animal as well as non-invasive imaging interventions on no more than 4 occasions which typically require anaesthesia and sometimes injection of a radiotracer (2-4 hours), contrast agent or light emitting compound. Typically these are spread over the several months or years lifetime of an animal. Animals may be culled under general anaesthesia.

Animals will typically be injected with a range of drugs/treatments and followed up for up to a year. Animals are always monitored closely throughout the length of the experiment to ensure good health and any signs of pain, distress or ill-health are addressed accordingly (pain-relief, antibiotics, creams, etc). At the end of the experiment the animals are humanely killed and organs are typically harvested for further analysis.

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

Rodents

Expected adverse effects include weight loss (weight loss will not exceed a humane endpoint of not more than 20% of starting bodyweight), pain following surgery, lethargy and abnormal behaviour. Animals can experience stress due to restraint and handling which will typically resolve by the end of the procedure. Sometimes food withdrawal for up to 16 hours prior to a test may be necessary for one or two behavioural tests which can cause stress, again limited to the duration of the test. Typically behavioural panels will be performed up to 3 times separated by several weeks. Where animals require anaesthesia, they will experience transient discomfort from needle insertion and/or anaesthetic injection or inhalation of gaseous anaesthetics. Animals may undergo anaesthesia on up to 4 occasions for imaging , up to 10 occasions for retinal imaging and once for intracranial therapy or tumour implantation. Sometimes, during surgery the animal’s breathing can seem laborious, this typically resolves by the end of surgery. Sometimes seizures or brain bleeds can happen in the initial hour after surgery. There is also potential for infections. Delivery of chemotherapeutics to mice (usually 5 days of drug) prior to bone marrow transplant will make mice quite sick, as is the case in patients with a depressed immune system and low blood counts for several days leading to lethargy and weight loss (weight loss will not exceed a humane endpoint of not more than 20%) during this period before the transplant takes.

Sheep

Some possible pain during/after surgery, indicated by teeth grinding. Occasional mild adverse effects of surgery, such as transient tremor or partial facial paralysis. Typically all of these resolve within 48 hours. Sometimes seizures or brain bleeds can happen in the initial hour after surgery. Anaesthesia on no more than 5 occasions; once for intracranial surgery once and four occasions for imaging. There is also potential for infection. Sheep are typically housed in pairs to avoid distress.

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)?

For mice, expected severities will be mild to moderate, In breeding protocols 50% of mice will experience subthreshold harms, 40% mild and 10% moderate. In experimental protocols 10% will be mild and 90% moderate. Overall, as most mice fall into breeding protocols - we expect 40% of mice to be subthreshold, 30% of mice to be mild, and 30% moderate. Ffor rats it will be mild to moderate – we expect 70% of rates to be mild and 30% moderate and for sheep it will be mild to moderate. We expect 10% of sheep to be mild and 90% of sheep to be moderate.

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?

The conditions we work on are complex and are influenced by a host of different factors. Many of these conditions are neurological in nature but also have non-neurological components often including heart problems, bone disease and eye degenerations to name a few. As such, non-animal alternatives, such as cell-based tests, may not be appropriate as they can only provide limited results. The mouse models of these conditions mirror the human disease closely, giving us physiological data, such as cell-cell interactions and immune system dysfunction, which cannot be achieved using cells in a dish, nor with zebrafish models. We need to assess that the therapies we develop have the ability to cure all of the affected organs, especially the brain, so computer based assays and cell culture cannot predict outcomes. There also are no appropriate methods to avoid the use of animals to assess cell engraftment after bone marrow transplant of the delivery of gene therapy vectors to the brain. Our therapies are assessed with a variety of outcome measures such as behaviour and tissue sampling which can only be achieved with animal models. When testing our therapies for direct treatment of the brain, it’s necessary to use an animal model whose brain is comparable to the human brain in size. This cannot be achieved by any other method therefore it necessitates the use of a large animal. Sheep are one of the least sentient of these.

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

We use cell-based tests in the lab to replace animals where possible (e.g. Cell culture assays, High Content Screening technology), which we use to reduce drug or gene therapy candidates to viable numbers for in vivo testing by aiming them at specific characteristics -such as blood brain barrier penetration and cell uptake. We have used agarose phantoms to model brain distribution, but this is

not very predictive of in vivo outcomes. There are no suitable in vitro assays or alternatives that are therefore relevant to distribution, delivery of products, immune system responses, brain and behavioural outcomes.

Why were they not suitable?

No adequate tissue culture models of the complexity required to examine tissue and brain repopulation after transplant in living organisms exist. In order to better predict what treatments or stem cells will prove to be effective and safe – we require animal models to test these approaches. The brain is incredibly complex, and we often use behavioural evaluation of mice with disease to assess the effect of therapy which is impossible in tissue culture and/or computationally.

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 based these numbers on our previous experience working with these models and the numbers used on previous licences. Around 40% of our rodents will be used in breeding only and most of our rodent lines breed as heterozygotes yielding the proportion of one unaffected, two mixed and one affected mouse - and the remainder will go into protocols. Typically we will maintain up to 5 rodent lines at any time, the remainder being kept as frozen stock.   For example, we have over a decade worth of experience performing bone marrow transplant experiments in mouse models of orphan diseases. From this, we can estimate that we will typically need 8-10 mice in each group to see differences between treated and untreated mice (by analysing the mouse's behaviour and markers in bodily fluids/tissue) and we would typically have 3-5 groups. We also have an accurate idea of how many experiments we would like to complete over the course of the 5 years that this licence will run. We expect to evaluate 10-12 new substances of which 2-3 will be taken forward to clinical trial.

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

We will use statistical methods to calculate the number of animals needed for our experiments. We will also perform as much work as possible in non-animal models, such as cell culture experiments, to further reduce the number of animals needed.

We often use repeat imaging to reduce the number of animals that we have to cull at set time points This means that we reduce wastage and allow paired data analysis to reduce between subject variation. We also usually share controls between experiments where possible by running multiple experiments in parallel.

We often use repeat imaging to reduce the number of animals that we have to cull at set time points and usually share controls between experiments where possible by running experiments in parallel. We have extensive experience of working with animals and performing these kinds of studies.

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

We will breed animals in such a way that the number of animals from each mating is optimised and we will collect data throughout the lifespan of the animal to generate the maximum amount of data and reduce the number of repeat experiments needed. Were possible we will utilise imaging and sampling techniques throughout the lifespan of the animal, reducing the need to sacrifice animals. We also have a number of collaborators, therefore, maximum use is made of animal tissues across a number of different projects.

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.

To achieve the objectives set out in this project licence we will need to breed a number of different mouse models. These will include transgenic mice expressing marker proteins (allowing tracking of various cells following transplantation) as well as the mouse models of mucopolysaccharidosis (MPS) type I, II, IIIA, IIIB IIIC, MPSVI, Wolman disease, Fabry, Krabbe and Gaucher. These are the best mouse models of these progressive childhood metabolic diseases available, are the least severe models, and are used in preference to naturally occurring cat and dog models of the diseases. Each generates a distinct disease and despite similar substrates stored display different behavioural and biochemical outcomes. Comparisons of these models will help us to understand which components are important in each diseases' progression. These models will be used to examine how the bone-marrow-derived cells travel to the brain and also to test the therapeutic effects of the cells, particularly gene therapy modified cells to express the missing enzyme.

Immunodeficient mouse models will be used to study how human stem cells can repopulate organs such as the brain. Each model knocks-out different immune cell types – allowing us to see which are important in aspects like tumour progression. These mice will also be used as they have the ability to grow human brain tumours when they are injected into the head. Disease specific models crossed onto an immunodeficient background, allow the use of human cells to treat the model.

We need to use a large animal model to scale therapies from mice to humans. This is because the mouse brain does not replicate the structure, blood flow or density of the human brain and therapies in mice sometimes dont predict accurate dosing or delivery routes for therapies. A sheep's brain is as

large as a large monkey and has a similar structure, blood flow metabolism and density to humans. It also shows lysosomal storage therefore it is the most refined model for this purpose, while being less sentient than monkeys.

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

Rodents are the least sentient species in which models for these orphan diseases exist and provide some of the best available models of human disease progression as we have characterised previously. Other models, such as zebrafish, are inappropriate as abnormal behaviour is a characteristic of the diseases we research and zebrafish poorly model these aspects. Although we could start by treating zebrafish to show proof of mechanism, this would not reduce the number of mice required to demonstrate behavioural changes when we deliver a therapy and we typically show that enzyme works in cell culture in advance of any mouse studies. Also it can be difficult to test therapies in zebrafish and, as many regulators do not allow studies in zebrafish prior to clinical trial, we would need to use mouse models anyway so the use of zebrafish seems an inappropriate use of animals. The brain of a sheep is structurally, in terms of blood flow and disease modelling closest to that of a human child compared to other large animal models and is the least sentient of these with large brain size and, therefore, will be used for scaling therapies from mice to humans.

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

We always look to improve the way we perform procedures. For example, we use chemotherapy to prepare mice for bone marrow transplant in place of irradiation, with much improved mortality rates (now less than 1%) and reduced adverse effects on health to mice overall. As a part of this project we are looking at other chemotherapeutics that may improve this further.

Our mouse intracranial surgery is extremely well tolerated (less than 1% mortality) and this is in part due to the efficiency with which we can complete the procedure given our experience with the model of the procedure (typically under 20mins).

We are able to use image guided sheep intracranial surgery using a CT scan of the sheep brain to create a 3D image of blood vessels prior to surgery and mapping to the skull of the sheep, which is another innovation from patients allowing safe planning, and significantly reduces the time and risks of intracranial surgery in sheep. We have a less than 5% mortality rate in sheep with this technique, which would be much higher without it.

All procedures are undertaken using good aseptic technique to minimise the risk of infection. Post-operative care will involve constant monitoring in the weeks and months following procedures. Animals will be weighed and assessed for pain and distress. Analgesia (pain relief) and other treatments will be used if necessary and in consultation with the vet.

We always keep up to date on the latest guidance and will undertake training when required to improve how we handle and perform procedures on animals.

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

All our experiments will be conducted following PREPARE guidelines, ARRIVE guidelines and OECD protocols.

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

We liaise with colleagues in the animal facility within the establishment who keep us up-to-date on advances in the 3R’s via a monthly newsletter. We also access the NC3Rs and other welfare driving bodies websites for training information. We will also seek to attend seminars and other educational events to further this knowledge as well as reading publications and outputs of colleagues.

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