Home Office

NON-TECHNICAL SUMMARY

# The development of novel therapies (including cell and gene based approaches) for Parkinson's and Huntington's disease

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research

**Key words**

Stem cells/gene, Novel therapy, Immunogenicity (the ability of cells/tissues to provoke an immune response), Parkinson's disease, Huntington's disease

| Animal types | Life stages |
| --- | --- |
| Mice | adult, embryo, neonate, juvenile, pregnant |
| Rats | 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's the aim of this project?**

1. There is currently no cure for Parkinson's disease (PD) and Huntington's disease (HD) or any way to stop it from getting worse. Our project is to show if new therapies, including gene and cell therapies, can improve symptoms and restore neural circuits in animal models of PD and HD.

2. Rejection is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted cells or gene. Our project is to understand how the human immune system respond to cell transplants and/or gene injections designed to treat aspects of PD and HD, then find ways to prevent them from being damaged by the human immune system.

**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?**

Parkinson's Disease is the second most common neurodegenerative disorder affecting approximately 1% of those over 60, and is expected to become increasingly prevalent in an ageing society. Currently available therapies provide symptomatic relief for some of the movement problems, but to date, there are no disease modifying therapies for PD. Huntington's disease is a rare genetic neurodegenerative disorder affecting approximately 2.71 people per 100,000 worldwide. It is a fatal condition that typically develops between the ages of 30 and 50 years old and progresses over a 20-year period. There is no cure for Parkinson's disease and Huntington's disease currently. It is important to find novel therapies for these patients that could better help treat their clinical features as well as slow down disease progression.

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

1. Publications and presentations. We anticipate publishing the results of these studies in peer-reviewed scientific journals. We also intend to present the findings of our work at local, national and international scientific meetings as well as to patient groups and at public meetings.

2. Novel data to support future funding applications.

3. We hope to use our data to design future clinical trials around our discoveries thus allowing for a translational aspect to the work. We are very motivated to develop clinically relevant potential therapeutic applications for patients with Parkinson's and Huntington's Disease.

4. The generation of new information around better understanding the neurobiological basis of Parkinson's and Huntington's Disease in vivo (studies done within living organisms).

5. The data produced by this project will potentially support patents application of these candidate therapies.

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

In the short-term these findings will benefit the ongoing research within the laboratory including developing new therapeutic agents and better understanding the immune response to gene therapies and transplants of stem cell derived products placed in the brain. In the medium-term this work will help to develop potential new therapies that could be used to treat Parkinson's and Huntington's disease as well as to better understand the need and type of immunosuppressive regimes required in such patients treated with gene and cell therapies.

In the long-term we hope that this work will lead to translational outputs in the form of clinical trials around new therapeutic agents.

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

Collaborating- we will work closely with groups who have complementary knowledge using humanised mice (mice lacking their own immune system but given a human immune system) as a model to study immunogenicity as well as with groups working on novel therapies including cell and gene therapies and ways of delivering these to the Central nervous system (CNS).

Disseminating new knowledge - this will be done through presenting our work including negative results at local, national and international meetings and by publishing our work in peer reviewed journals as well as presenting findings to patient and public groups as appropriate.

**Species and numbers of animals expected to be used**

- Mice: 3350
- Rats: 500

# 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.**

1. We will use adult mice wild type littermates and sham transplants as appropriate for controls in our experiments. This will allow us to ascertain whether any effect seen during the experiments carried out is a true one and will thus avoid any false positives.

2. We will use adult immune-compromised mice that have been given a human immune system (humanised) to address the immune response of human cells within the mice in response to transplantation of stem cell derived products or gene injections. Humanisation is when human immune cells are introduced into the mice (by injection), they can survive and form a working immune system. This process is called engraftment. We have extensive experience of working with immune-compromised mice and know that they are the best type of mice to use for these sorts of experiments.

3. We will use adult transgenic mice as a model for studying Huntington's disease. We will use these mice to better understand the effect of novel therapies including cell and gene therapies in these diseased animal models.

4. We will use adult rats to model Parkinson's disease via a novel delivery system to transfer abnormal protein aggregates across the blood brain barrier and explore the possibility of validating existing or novel therapeutics. In short, preformed fibril (pff), a type of abnormal protein aggregate, will be injected intravenously then the pff will get into brain by penetrating the blood brain barrier and gradually cause some brain damages similar to Parkinson disease patient's. We will also use adult rats to model Parkinson's disease by injecting locally into the brain with neurotoxin such as 6-OHDA.

5. We will use adult rats to model Huntington's disease by injecting locally with a virally delivered mutant Huntingtin (mhtt) construct. By injecting mhtt expressing virus into the brain, we are hoping to make a Huntington's disease model.

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

Immunodeficient mice may undergo a combination of the following procedures depending on the experimental design.

We will use adult mice that lack an immune system of their own (such as NSG mice) to answer "To demonstrate the immunogenicity of transplanted cells/genes in humanised animal models".  Because these mice lack an immune system, they can be "humanised". That is - when human immune cells are transplanted into the mice (by injection), they can survive and form a working human immune system. This process is called engraftment.  We have extensive experience of working with NSG mice and know that they are the best type of mice to use for these sorts of experiments.

To create the PD model, we inject a neurotoxin into the adult mouse/rat brain to mimic the damages in the patient's brain. This process is called lesioning. After lesioning, animals will undergo behavioural testing (such as walking on balance beam and grip strength) to find out if their behaviour is impaired, i.e. they have the symptoms of PD. If the disease modelling is successful, cell transplantation or gene injection (cell/gene therapy) will be carried out on those animals, aiming to reduce/eliminate the symptoms, which will be demonstrated by repeating the behavioural testing up to 6 times. Mice will receive one (for lesioning) and/or a second (for cell transplant) anaesthetics.

R6 mice are a transgenic line that carries part of a mutant form of the human Huntington gene and can be used to model HD. When R6 mice start to show symptoms of HD, they will undergo a battery of behavioural testing before and after the cell transplantation or gene injection to investigate any effects of these therapies. The behavioural testing will be repeated up to 6 times.

Rats may undergo a combination of the following procedures depending on the experimental design:

Rats will be injected intravenously (on a rare occasion up to three iv injections each under anaesthetic) with a type of abnormal protein aggregates (pff) or locally injected intracerebrally with neurotoxin or a virally delivered mutant Huntingtin (mhtt). Again, a battery of behavioural testing will be performed before and after injection to enable comparison of the effects of therapies. The behavioural assessments will be repeated maximum 6 times and the majority of rats will receive 2 anaesthetics (one for disease induction and one for injection of cells/genes).

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

1. An estimated 20% of mice of strain NSG will show swelling around the hocks. Any animals experiencing swelling around their hocks may be given an altered enrichment, pain relief and/or anti-inflammatories in consultation with the NACWO and/or NVS.

2. No or minimal adverse effects are expected from transferring a human immune system (humanisation) of these animals other than the transient discomfort from the injection. Graft versus host disease (GvHD) is something that will occur over time and will be watched for as a side effect of the humanisation procedure. We have a great deal of experience of the time course of this happening.

3. Following stereotaxic injections (three-dimensional surgical technique that enables precise injection deep in the brain) of toxins, gene therapies or stem cells, our experience is that this induces no harmful lasting side effects, with animals returning to normal after recovery. The following adverse effects are expected post-operatively: animals may be subdued but show progressive improvement in response to stimuli. Over the period 24-96h, animals may show reduced appetite for food and water, which is expected to result in minor weight loss (less than 5-10% of their pre-operative weight) within this 4-day period. Minor dehydration may therefore also occur and will be alleviated by addition of mash or gel to the cages.

4. For the rats used in the procedure involving intravenous administration of abnormal protein aggregates, this will be conducted under general anaesthesia, which normally last between 5-10 minutes. Due to the short time that these animals will be under anaesthesia, we expect them to recover quickly with no more than transient discomfort and without lasting harm from the anaesthetic procedure itself. From 6 months after the initial intravenous administration of abnormal protein aggregates, it is possible that some animals may start to develop Parkinson's disease-related phenotypes, such as deficits in the olfactory (the sense of smell), motor, and cognitive functions. As a result, animals can show signs of reduced activity, abnormal gait (manner of walking), as well as changes in body weight. While we hope such deficits to slowly progress over the next few months (up to 15 months of age), they will not cause any pain to the animals, and will not interfere with their ability to move, drink or eat freely.

5. R6 mouse strains are expected to progressively show adverse effects associated with a mild to moderate severity category. The majority (~80%) of animals bred and maintained under this protocol are expected to remain within or below the mild severity criteria. Approximately 20% of all animals are expected to develop a neurodegenerative phenotype reaching moderate severity.

**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)?**

Mice

Mild-10%

Moderate-90%

Rats

Mild-10%

Moderate-90%

**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?**

Neurodegenerative diseases such as PD and HD are associated with widespread pathology and many neurological and cognitive deficits, necessitating whole system animal studies, although these can be supplemented by techniques such as in vitro (performed in a test tube, culture dish or elsewhere outside of a living organism) slice cultures. Some information about the networks that underlie specific clinical aspects of the disease can be gathered from the patients, using techniques such as functional imaging as well as post mortem studies but trying to treat them necessitates studies in animal models.

Animal models are needed to study causality and disease progression in ways unavailable with patients. For example, we cannot interfere with the drug regimens used to treat patients clinically nor can we undertake precise mechanistic and therapeutic studies with novel agents without recourse to animal models to show survival, safety and functional efficacy.

Thus the experiments undertaken in the licence can really only be done in animal models of disease given that we plan to: (a) mimic the clinical scenario as closely as possible in the complex mammalian brain and how it responds to agents that are designed to work by either rescuing or replacing networks of degenerating neurons; (b) study the efficacy of therapies either across the pathology of the whole brain or in selective populations and networks of connected cells; (c) study the effects of therapies on a range of animal behaviours over time including motor and cognitive deficits; (d) document the possible side-effects of any such therapies including weight changes, etc and e) show that the cell and gene therapy survives and innervates or distributes over the volume of brain needed for patients.

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

Growing stem cells derived from patient's skin biopsies will allow in vitro studies to replace some that involve animal tissue, and developments in biochemical analysis of patient's samples such as blood and cerebrospinal fluid are yielding more information, supplementing that from animal studies. Some in vitro modelling using embryonic stem cells and relevant derivatives have been used to study human immune responses along with in vitro human foetal tissue studies for testing new potential therapies.

**Why were they not suitable?**

This in vitro work is a useful starting point but ultimately cannot be used to study how the therapies work within the diseased brain and affect the systemic human immune system. This can only be done using animal models of disease and humanised mice.

# 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?**

This number has been estimated from our extensive experience of doing this kind of work. We have estimated that 350 mice will be required for "rederiving" (i.e. establishing a new colony of mice from frozen eggs and/or sperm) any further strains of mice we need for this project. Up to 3000 mice will then be used to answer the experimental questions.

We will use up to about 500 wildtype rats for experiments on Parkinson's and Huntington's disease model.

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

During the experimental design phase, we (i) sought guidance from the NC3R's experimental design assistant on-line tool and (ii) reached out to collaborators who have extensive experience of the specific animal models we are using. In addition, we have in house experience of neurotoxic lesioning and cell transplantation, and our number estimates are based, in part, on these data. Our experimental design has also been informed by effect sizes seen in vitro and similar work done in the past or published.

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

To minimise animal usage, we will:

● Breed animals as efficiently as possible - attempting throughout to match breeding with experimental needs as closely as possible. This will require careful experimental planning.

● Throughout the project we will perform our experiments on small groups of animals (3-5 animals at a time), in pilot studies, and will review the results prior to extending our work to larger numbers and thus we will adapt our work accordingly.

● Share animals with local collaborators - this is particularly relevant for NSGs where there are a number of groups locally using this animal model. This is something we routinely do on our current licence.

● We will consider placing mice into experimental groups randomly and that treatments are given 'blind'. In that way, the experimental results are analysed blindly as well to avoid any bias when drawing conclusion.

● In certain experiments we could do bilateral stereotaxic surgery placing the experimental stem cells in one side of the brain and a sham injection on the other side such that a mouse could act as it's own control. This will reduce the numbers of mice being needed for certain experiments.

# 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.**

Transgenic mice will be employed in some studies as they contain the genes responsible for unique human disorders, such as HD. This enables studies which more completely recapitulate the pathology and behavioural deficits of the human disorder.

PD or HD rat/mice models can be also achieved by acute surgery. Acute surgical lesioning may involve more than one lesion (e.g. bilateral injections), but this is limited to a single surgery session of lesioning. Acute lesions may actually have either an acute effect (whereby lesions are created rapidly over a short period of time) or a progressive effect (whereby lesions develop slowly over time in response to an initial insult) mimicking the progressive nature of PD and HD neurodegeneration. An example of an acute lesion is one created through the administration of neurotoxin which takes almost immediate effect and for instance, aims to irreversibly remove the dopamine input to a certain part of the brain akin to that seen in advanced PD. An example of a progressive lesion is through the administration of viral vectors by a single injection to the brain, which leads chronically over time to the progressive accumulation of pathological proteins in cells and so mimics the progressive cell loss.

Other experimental work can be best done in rats (particularly cellular therapy strategies) given the long history of their use in our studies and the better described behavioural repertoire. Typically, the lesions are either acute or chronic and the animals display specific behavioural deficits that will be ameliorated if the novel therapeutic including dopamine cell therapies work to restore or reverse the pathology seen in these models. For example, from 6 months after the initial intravenous administration of abnormal protein aggregates, it is possible that some animals will start to develop Parkinson's disease-related phenotypes, such as deficits in the olfactory, motor, and cognitive functions. As a result, animals can show signs of reduced activity, abnormal gait, as well as changes in body weight. While we hope such deficits to slowly progress over the next few months (up to 15 months of age), they shall not cause any pain to the animals, and shall not interfere with their ability to move, drink, or eat freely.

We will use adult mice that lack an immune system of their own to study the immunogenicity of novel therapies by humanising them. That is - when human immune cells are transplanted into the mice (by injection), they can survive and form a working human immune system. This process is called engraftment.

**Why can't you use animals that are less sentient?**

Rodent models are the least sentient organism still able to reasonably mimic the human neurodegenerative diseases we are studying, with a brain which is anatomically similar to the human brain. Mice and rats differ both in their behavioural capacities and in the experimental practicalities as a consequence of their differing brain sizes; although the basic approach remains similar for both species.  Which species is used in each experiment will be determined to ensure that we maximise the probability of obtaining clear, valid and reproducible results whilst causing minimum distress to the animal.

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

All animals used on this project will be monitored daily for any signs of ill health or distress. Particular care will be taken after surgery, with increased frequency of observation over the first few hours post-operatively, and through the use of pain medication which could be provided in the way of flavoured jelly (or such-like) that can be self-administered by the mouse. Immediately post-operatively mice will be kept warm and then will be housed in cages with additional soft bedding.

Throughout our project, we will aim to improve the quality of life for our animals – for example by providing enriched environment, acclimatisation and housing mice in social groups whenever possible and by avoiding single housing (apart from in exceptional circumstances, which will be time limited). Any animals experiencing swelling around their hocks may be given an altered enrichment, pain relief and/or anti-inflammatories in consultation with the NACWO and/or NVS.

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

We are aware of, and will always aim to adhere to, good practice guidelines (as given by the Laboratory Animal Science Association; LASA). We will ensure that we follow the LASA guidelines for performing aseptic surgery (as given in the updated 2017 document) – such as writing a pre-operative plan, administering pain medication and fluids as required and most importantly ensuring that the procedure is performed by a researcher who is fully trained.

We are also aware of the PREPARE (Planning research and experimental procedures on Animals: recommendations for excellence) and ARRIVE Guidelines (Animal research: Reporting of in vivo Experiments, version 2.0). PREPARE guidelines are for planning animal experiments as improved reporting, although important itself, can't improve the quality, reproducibility and translatability of animal studies. These guidelines are designed to complement ARRIVE guidelines that are a checklist of information to include in publications describing animal research. It includes their reporting guidelines around the importance of information on experimental design and statistical analyses and strategies to minimise bias and how to report in publications.

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

We will remain informed about advances in the 3Rs by speaking to colleagues locally (including veterinary surgeons, senior animal house technicians etc) and by reading information sent to us by the local licencing department. We will also ensure that we stay up to date by visiting relevant websites (including that of the NC3Rs and Norecopa) and by attending relevant conferences and training events.

As a project team we will meet regularly to ensure that we are adhering to the 3R guidelines, and implementing any changes effectively.