G: NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed project clearly using non-technical terms which will be understandable to a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary may render your application incomplete and lead to it being returned.

(WORD LIMIT: 1000 WORDS)

Please complete the following:

<table>
<thead>
<tr>
<th>Project Title (max. 50 characters)</th>
<th>Experimental Molecular Medicines for Restorative Neuroimmunology</th>
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<tbody>
<tr>
<td>Key Words (max. 5 words)</td>
<td>Molecular therapies, inflammation, brain, stem cells, brain repair</td>
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<td>Expected duration of the project (years)</td>
<td>5</td>
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<tr>
<th>Purpose of the project as in ASPA section 5C (3) (Mark all boxes that apply)</th>
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<tbody>
<tr>
<td>X Basic research</td>
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<td>X Translational and applied research</td>
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<td>Regulaory use and routine production</td>
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<td>Protection of the natural environment in the interest of the health or welfare of humans or animals</td>
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<td>Preservation of species</td>
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<td>Higher education or training</td>
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<td>Forensic enquiries</td>
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<tr>
<td>X Maintenance of colonies of genetically altered animals¹</td>
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Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)

Treatment options for patients with central nervous system (CNS) disease are currently limited, and many CNS disorders still do not have a cure. Despite years of research in the field of CNS injury, this persistent lack of therapeutic options has prompted the scientific community to re-evaluate the mechanisms underlying the pathophysiology of these diseases. In the past few years, it has become increasingly clear that many of the events that characterize CNS injury are profoundly intertwined with the activation of the immune system, which guides most of the degenerative and reparative processes. The new direction of research (both in Cambridge and worldwide) is to treat CNS damage (either acute or chronic neurodegenerative) by optimally engaging and modifying these immune responses (immunopathobiology).

Our project's aim is to understand the mechanisms underlying immune system activation in CNS disorders, in order to enable the identification and validation of new drug targets, with the ultimate goal of developing novel therapies.

¹ At least one additional purpose must be selected with this option.

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What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

| What species and approximate numbers of animals do you expect to use over what period of time? | Mice: 9,400 in 5 years.  
Rats: 1,650 in 5 years. |
|---|---|

Treatments for CNS damage are currently limited, and once the clinical deficits are established, it is almost impossible to revert them. This leads to massive costs for the long term care of patients and a gigantic burden for their families and the NHS. Within this project we purposely focused our attention on the three major CNS disorders that are characterized by the highest prevalence, the uppermost degree of long term disability and the lack of any real restorative treatment.

Spinal cord injuries (SCI) – It is estimated that almost 40,000 people in the UK are living with SCI, with approximately 1,000 new cases each year. Most of the injured people are young adults between 20 and 35 years old and, although modern medicine has allowed them a normal life expectancy, the lack of any regenerative therapy results in massive costs related to long-term medical support (the cost to the nation is estimated at £1 billion per annum).

Multiple sclerosis (MS) – The onset of MS is typically between the ages of 20 and 40 (female: male ratio is 3:1), and it is calculated that in the UK alone (which is a high rate country for MS), more than 100,000 people are afflicted by this disease. As a matter of fact, MS is one of the most common causes of chronic neurological disability of the early-to-middle adult life, and it is calculated that the associated costs (direct and indirect) in the UK are about £1.5 billion per annum. Current available therapies for MS contribute to these expenses (being very expensive), but in the end they are only partially effective to limit the inevitably progression of disease.

Ischemic stroke – Stroke constitutes a major health problem in UK, being the fourth largest cause of death (after heart disease and cancer), the second cause of dementia and the primary cause of long-term disability in the UK. In figures, stroke kills twice as many women as breast cancer and more men than prostate and testicular cancer combined a year. The economic costs of stroke in the UK are about £9 billion per annum, and while there is only one specific treatment for acute stroke (thrombolysis, which is applicable in only 5-10% of stroke patients), there are no effective treatments to revert established stroke deficits.

Within this project we are proposing an original approach to these diseases that focuses on the study of the contribute of the immune system in mediating CNS damage and halting recovery. By studying the pathways involved in inflammatory-mediated CNS damage, our aim is to develop highly innovative therapeutics to help overcome limited efficacy of existing approaches.

Despite the severity of the protocols herein proposed, our approach will bring to massive benefits for the NHS (reduced costs), the patients and their families (better quality of life). Moreover, the use of our basic science approach will help the scientific community to advance its knowledge on the biology of CNS diseases, as well as to develop novel methods, technologies, and protocols.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

In this project we are proposing to use several genetically altered animal (GAA) models to study the role of inflammation in brain repair. None of the proposed GAA is expected to have a detrimental phenotype.

A second approach will be to study the immune system by creating bone marrow chimeric mice. In this case, the immune system of the animals will be replaced by other immune cells, which have been previously manipulated in vitro. This approach has several adverse effects related to the transient immune suppression of animals (e.g. increased risk of infection).

A third approach described herein is to use in vivo preclinical models to study the pathobiology of immune cell activation in specific CNS diseases.

In this case, for modelling SCI we will use the contusion injury of the spinal cord obtained via a sophisticated computer-assisted impactor. This device provides well-calibrated and reliable readouts, and it allows fine control of tissue displacement and measurement of the actual impact force. Rodents will initially develop a paralysis (reduced strength) and impaired movements of both hind limbs, which will spontaneously recover within the first few weeks post-surgery (recovery depends on the force used to induce the contusion).

For MS we will use the experimental autoimmune encephalomyelitis (EAE), which is a well-studied immunological model characterized by the subcutaneous injection of myelin proteins capable of inducing the formation of immune cells that will attack the CNS. Mice will develop an ascending flaccid paralysis, which begins within the tail muscles, and then progresses to the hind limbs and ultimately the forelimbs. Usually the peak of symptoms is reached within 72 hours from the onset, and the severity of these clinical signs is sex, age, and strain specific.

For stroke we will adopt two standardized surgical procedures: the proximal middle cerebral artery occlusion (MCAO) approach (in which a silicon coated filament is introduced in the arteries of the neck and pushed towards the MCA to occlude it) or the distal MCAO approach (in which the distal branch of the MCA is occluded after drilling a small hole in the cranial of the rodents). These approaches are used to model major ischemic events or small cortical infarcts, respectively. In both cases, rodents are expected to develop a hemiparesis (loss of strength) on the opposite side to the stroke.

Rodents subjected to either of these models will be treated with novel therapeutics aimed at modulating the activation of the immune system. These will include the local implantation in the spinal cord of ACTIVE MULTIFUNCTIONAL IMPLANTABLE ORGANIC DEVICES (for SCI), the transplant (either systemically or locally) of STEM CELLS AND EXTRACELLULAR MEMBRANE VESICLES (EVs) derived from stem cells, or the injection (either systemically or locally) of innovative NANTHERAPEUTICS.
Application of the 3Rs

1. Replacement
State why you need to use animals and why you cannot use non-animal alternatives

Since no experimental treatment could be ever tested in human patients without extensive and appropriate validation in relevant animal disease models, concepts developed in tissue culture have to be tested and refined in vivo where the complex environment of the adult nervous system is present, and where functional recovery can be measured. Both mice and rats are widely used in research because their genetic, biological and behavioural characteristics closely resemble those of humans, thus allowing the study of many of the pathologies (or part of these) seen in patients. In particular, the possibility to use transgenic mice in which the expression of specific genes can be monitored and/or altered allows the study of target proteins and pathways involved in CNS injuries and repair. Moreover, Immune-compromised mice are extensively used to host and test the safety of allogeneic transplants. Within this license we propose the use of different animal models to mimic three major human diseases (namely SCI, MS and ischemic stroke). However, before proceeding with animal work, all our new interventions are tested and refined in tissue culture. In case of cellular therapies and cell-derivatives (e.g. EVs), we routinely test measures that include the estimation of growth rate, differentiation ability, and negative screenings for pathogens (e.g. mycoplasma). For all candidate nanotherapeutics and AMID, the immunogenicity of the preparation will be tested in vitro first on relevant cell lines. Finally, all these interventions are tested for efficacy in vitro on microglia, macrophages, astrocytes and neurons prior to in vivo testing. To further ensure the significance of the results observed in vitro, all the experiments are run in multiple technical and biological replicates.

2. Reduction
Explain how you will assure the use of minimum numbers of animals

In our studies we always try to minimise the number of animals used while ensuring the achievement of sufficient data to properly answer a specific research question. The efficacy of the proposed therapeutic will be evaluated via a combination of behaviour, imaging, neurophysiology and conventional pathology approaches. The use of these combined readouts is part of our reduction approach as it allows to gain serial measurements from the same animal, avoiding the need of further experiments, with the final aim of maximizing the amount of data that can be obtained from a single animal without compromising animal welfare. For this reason, we have adopted the use of non-terminal anaesthesia (where possible) to guarantee proper biological fluid sampling, electrophysiological monitoring, and imaging of the same animal over time. This will provide better information about the progression of the disease/therapy while reducing the number of animals used. In our lab we have been extensively using SCI, EAE and MCAO models in rodents (approximately 60 published studies over the last 10 years) and we are therefore able to ensure a high rate of reproducibility (consistency of immunopathology, neuropathology, lesion size, and behavioural/disability outcomes). The animal group size for our experiments is based on our previous experience and statistical power calculations, so that the number of animals is sufficient to achieve statistically significant results. However, pilot studies will always be performed for new treatments (e.g. to assess feasibility, monitor potential side effects, and refine the main outcome measures of the experimental paradigm), and the number of animals to include in these pilot studies will be kept to a minimum (usually 5 per experimental treatment group).
3. Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Both mice and rats are widely used in research because their genetic, biological and behaviour characteristics closely resemble those of humans. However, in some cases there may be some important differences between mice and rats. For this reason and in accordance with the specific experimental purpose, in the under this PPL we will be using either mice or rats.

Modelling SCI: SCI is a complex pathophysiology characterized by different aspects, such as acute and delayed inflammation, cell death, demyelination and astrogliosis. Traumatic SCI represents the most common cause of injury in humans (45% incidence). Thus, the most appropriate model that mimics the human condition is the contusion SCI. There are two main reasons that support our choice of this model: (i) it mimics both anatomically and pathophysiologically the human development of this pathology. (ii) Contusion SCI selectively targets the region of the dorsal spinal cord hosting the corticospinal tract. Rodents, compared to the other species, allow the generation of a reproducible injury model, one of the crucial points for screening therapeutic approaches and treatments. This model has the advantage of allowing researchers to couple CNS pathobiology (e.g. white matter damage, inflammatory response, and cell engraftment in case of transplant studies) with neurophysiological outcomes. Moreover, compared to other less severe models of CNS damage used to study nerve regeneration (e.g. optic nerve transaction) this model permits the measurement of quantifiable behavioural deficits which are pivotal to monitoring the efficacy of our interventions.

Modelling MS: to efficiently model MS (and its inflammatory driven pathobiology), we are currently adopting the EAE model in rodents. It is important to say that this model is not MS, but it recapitulates most of the mechanisms leading to MS. EAE is the prototype for T-cell-mediated autoimmune diseases and it is induced by immunizing the host with myelin specific antigens. This generates a peripheral immune response which ultimately leads to immune cell infiltration in the CNS with consequent damage. The EAE model has the advantage of allowing researchers to study neuroimmune interactions alongside with behavioural deficits that can closely resemble both the first (relapsing remitting) and second (progressive) phase of MS. This is important if we compare EAE with other less severe models of CNS demyelination (e.g. cuprizone induced, lyssolecithin or ethidium bromide injections), which are characterized by a toxic damage to the myelin with little (or no) behavioural outcome.

Modelling ischemic stroke: in order to model ischemic stroke, and the complex ischemic cascade, in vivo non-reductionist approaches are needed. We are currently adopting the MCAO rodent model to mimic major ischemic events. This model has the major advantage of being highly reproducible (compared to the cardioembolic model of stroke), of best mimicking the pathophysiology of the ischemic disease (contrary to the phototrombic model), and having observable behavioural deficits. Refinement procedures include maintaining the mouse at internal temperature between 30.0°C and 37.5°C with a feedback heating system, as well as measuring the local cerebral blood flow with laser Doppler flowmetry. Both these approaches help reducing lesion variability and interpreting results.

Upon disease induction and treatment animals will be observed closely and, at the first sign of distress (e.g. animal failing to feed, drink, drop in the body weight) or pain/inflammation, the NVS will be contacted for a further advice. If animals show unexpected signs, or existing adverse effects will not respond to the treatments suggested by the NVS, animals will be killed.

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<th>For Office Use Only</th>
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<tr>
<td>Will the project be subject to Retrospective Assessment?</td>
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Version 1.5

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