



Home Office

## NON-TECHNICAL SUMMARY

# Immune and inflammatory mechanisms in cerebrovascular disease

### 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

### Key words

stroke, vascular dementia, inflammation, small vessel disease, brain

### Animal types

### Life stages

Animal types	Life stages
Mice	embryo, neonate, pregnant, adult, juvenile, aged
Rats	embryo, neonate, juvenile, pregnant, adult

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

### Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

## 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?**

To understand how inflammation contributes to cerebrovascular disease (stroke and cerebral small vessel disease) and to develop new treatments to reduce the impact of these conditions.

**A retrospective assessment of these aims will be due by 05 January 2028**

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

**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?**

Stroke, caused by a reduction in blood supply to the brain, is one of the leading global causes of death and disability, survivors being frequently left with significant complications such as vascular dementia, depression, anxiety as well as physical impairments. Cerebral small vessel disease is a term which is used to describe a range of conditions that affect the functioning of the small arteries in the brain, making them not work as well as they should, thereby affecting brain function. Cerebral small vessel disease is a very common condition particularly in older adults, causing up to 45% of dementia cases worldwide and accounting for approximately one quarter of all strokes.

At present there are no widely effective treatments for stroke and small vessel disease. Therefore, it is important that more research is carried out to develop these much needed therapies to improve survival and quality of life for people with cerebrovascular disease.

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

On completing this project there will be several outputs. These outputs include new knowledge about exactly how inflammation contributes to stroke and small vessel disease. Such knowledge will be shared beyond the research group through various means, including scientific publications, presentations at meetings, social media and dedicated websites. Importantly, our research will have a strong Patient, Carer, Public, Involvement and Engagement (PCPIE) aspect. We also expect to have

developed new drugs that could be potential stroke treatments or to have provided evidence to support the re-purposing of existing drugs for the treatment of cerebrovascular disease.

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

Ultimately the hope is that stroke and small vessel disease patients benefit from this research, due to the availability of new treatments. We have a proven track record in this regard, our previous similar research having led to current Phase 3 clinical trials of an anti-inflammatory drug in a subtype of stroke. Some of the current work we expect to similarly move to clinical trial, possibly within three years. The timescale will be dependent on whether new drugs are identified, or repurposed drugs proven effective, the timescale for developing the latter for use in patients being quicker.

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

We have a proven track record of progressing treatments along the translational pipeline, that is from studies in the laboratory through to testing drugs in stroke patients. To maximise the chances of doing this again from our current work we will work with relevant stakeholders. This will include patients and carers to ensure our research is relevant. We will also work with experts in our organisation with experience of developing new drugs, providing links with industry and investors, which is important in providing the support needed to progress the work towards use in patients. At all stages in the work, we will disseminate the findings, positive or unsuccessful, in relevant scientific journals that are freely available to everyone. We will also share our findings through collaborative networks and other appropriate routes, ensuring the widest possible reach.

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

- Mice: 4620
- Rats: 1480

## **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.**

Our work is focussed on deficits in the function of blood vessels in the brain and the reduced supply of blood that result in cerebrovascular disease (stroke and small vessel disease). The correct supply of blood to the brain to allow brain cells to work properly is referred to as neurovascular coupling. Importantly, neurovascular coupling in rodents is comparable to humans. Rats and mice also show similar damage to the brain when blood flow is reduced and animals develop complications like those seen in humans, including movement problems, cognitive decline, depression and other symptoms. In addition, mice and rats with risk factors for small vessel disease such as hypertension show clinical features of the human condition. The proposed studies could not be undertaken entirely in less complex animals such as flies and worms because they do not show such similarities to humans.

We will mainly use adult animals, though some of our studies in small vessel disease will use older (12-18 months of age) animals. The use of these older animals is important given the strong association between age and cerebrovascular disease in humans.

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

In order to mimic human stroke, we will use experimental procedures to modify blood supply to the brain in mice or rats. To modify the blood supply and mimic stroke the neck of animals will be opened through a small incision in the skin/muscle to reveal the carotid artery. The carotid artery is one of the main ways that blood gets from the heart to the brain. Then, using one of a few different approaches, we will stop or reduce the blood flow in the artery. Alternatively, we will make a small hole in the side of the skull to directly expose the middle cerebral artery. This artery is the most affected in human stroke and is therefore clinically relevant. We will reduce the blood flowing through the middle cerebral artery, using one of a number of different methods e.g. applying a small clip. We may also affect blood supply to part of the brain by injecting a dye that, when exposed to light, causes damage to the walls of the artery. This damage leads to a blood clot forming and a reduction in blood supply. This approach to induce stroke ('photothrombotic') can require the surface of the skull to be exposed. As well as stroke that results from blood clots (ischaemic) we are also interested one of the other types of stroke, intracerebral haemorrhage. To mimic brain haemorrhage in rats and mice we will directly inject into the brain very small amounts of substances that cause blood vessels to burst.

To model human small vessel disease, we will use mice and rats with relevant risk factors/genes associated with SVD, such as hypertension.

Stroke and small vessel disease are interlinked and strongly associated with different risk factors or co-morbidities. These risk factors/co-morbidities include hypertension, obesity, diabetes, atherosclerosis, and infection. To best mimic the clinical situation, it is very important that our experimental studies include these risk factors. Hence, in some of our studies we will use hypertensive, obese or diabetic mice or rats, and/or animals where infection is induced.

For all the surgical techniques described animals will be fully anaesthetised and will receive drugs (analgesics) to minimise any pain due to the surgery, as well as local anaesthetics at the wound site. We expect most of the animals to fully recover from surgery within an hour or two. The actual surgical procedures will typically last less than an hour, though this is dependent on how long the artery is occluded for, and the experience of the surgeon.

In some of our studies animals will undergo tests of behaviour. These behavioural tests are designed to assess any problems with movement or sensation as would be seen in stroke patients, or thinking problems as seen in small vessel disease, as well as other complications commonly reported by patients, including fatigue and depression. None of the behavioural tests are harmful to the animals and often just require observation for a short period (5-10 minutes) in specialised apparatus. Baseline tests before surgery will often be performed, with repeat testing at different times after surgery (up to 6 months). We will also assess the behaviour of animals with risk factors for small vessel disease as they develop symptoms over time, like the situation in humans.

Occasionally we will re-anaesthetise animals and use specialised imaging techniques to monitor changes in the brain that are important in cerebrovascular disease e.g. blood-brain barrier breakdown,

cerebral blood flow, inflammation etc. Such imaging may be repeated several times and be used to see if any drug treatments are working or not. These drug treatments will typically be designed to modify the effects of the stroke and/or reduce small vessel disease. These drugs can be given via various routes, for example a simple injection under the skin, or directly into the blood. On occasion we may want to give repeated drug treatments, thus animals will receive regular injections. Wherever possible we will try and reduce the need for such repeated injections, by giving the drug in the drinking water or food.

At the end of experiments animals will usually be killed by overdose of anaesthetic and we will take blood, brains, and other organs/tissues to investigate various measures that will help us meet our overall aims.

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

Clinically, stroke and small vessel disease are devastating conditions, resulting in significant mortality and morbidity in patients. In trying to model these diseases in animals a balance must be struck therefore between establishing a valid model and in minimising pain, suffering, distress, or lasting harm to the animal.

Stroke leads to brain injury and therefore there are likely to be behavioural effects on the animals similar to those seen clinically i.e. hemiplegia and muscle weakness, reduction in sensory or vibratory sensation, contralateral paralysis or weakness, listlessness, loss of appetite and loss of balance or orientation. However, these well-described behavioural changes are generally limited to the first day or two after the stroke. The impact of stroke, both in humans and in experimental models, is determined by the amount of brain damage. This can vary clinically but can be controlled in animals depending on the method used to induce the stroke. In many of our studies we will use approaches that cause only modest damage to the brain and therefore only modest behavioural changes. At all times it will be our aim to reduce any excessive suffering or pain experienced by the animals and to apply appropriate humane endpoints if an animal shows persistent adverse events.

Symptoms of small vessel disease are not as severe as acute stroke and therefore the models of small vessel disease that will be used do not show the same impact on behaviour. Small vessel disease animals will show modest changes in cognition, affecting how they perform in certain behavioural tasks. Such impairments should not affect their everyday ability to groom, socialise, feed and drink.

During a study animals can be exposed to several different interventions which may affect their behaviour and result in adverse effects. Drug treatment will regularly be used which will require injections or oral dosing. This may involve mild discomfort while animals are restrained and transient brief pain on needle insertion. Similarly, blood samples may be taken while animals are conscious, requiring brief restraint and the use of fine gauge needles/cannula to sample blood. There will be transient pain associated with the insertion of the needle. Ear clipping is performed only on genetically modified animals and on one occasion, therefore a small percentage of animals will be affected, with transient mild pain. Mouth swabbing and hair sampling are used rarely with only mild transient discomfort due to restraint and the sampling process.

In some studies, we will use animals with stroke-related comorbid disease, including hypertension, infection, and metabolic syndrome (obesity/diabetes). The hypertension is only mild and does not impact on animal welfare. Induced infections are mostly sub-clinical and therefore no obvious adverse

effects are observed, aside from subdued behaviour for a brief period. Diabetic animals may urinate more frequently and require additional fluid and more regular cage cleaning and/or provision of more absorbent bedding material. Over time, obesity may lead to insulin resistance and low-grade inflammation, like that observed in individuals with type 2 diabetes. These effects of obesity may cause subtle changes in behaviour (e.g. reduced activity), but are not associated with any lasting suffering or distress.

To account for the effects of surgery and other experimental interventions we will often include a sham group in our studies, which is normal practice. These sham animals will experience the process of surgery and as many of the same interventions as the experimental group as possible, short of the actual process being studied i.e. induction of stroke. Where we require accurate administration of substances into the brain animals will be secured in a special (stereotaxic) frame. They will be anaesthetised and no adverse effects of being placed in the frame are expected.

### **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)?**

Although we will always adopt the least severe approach necessary to meet the objectives, the devastating consequences of stroke on a patient could be mirrored in any model designed to closely mimic the clinical condition. Therefore, adverse events are on occasion possible, including death. The latter though is not expected to occur in any >1% of animals and only with certain stroke models. We will use our extensive experience of the models to be used to identify any animals at risk of showing a decline in condition that might lead to death and intervene before this occurs. Many of our studies will be designed with treatments that aim to reduce the amount of damage and therefore any adverse effects will be lessened in such experiments.

The greatest severity level experienced by animals will normally be due to the amount of brain damage. Hence, where stroke is caused by blocking one of the larger vessels to the brain or by causing the rupture of blood vessels then there is the possibility of severe suffering. However, we will most often occlude the artery at a higher point in the brain, leading to less damage which usually means the animals show moderate changes in behaviour, or even mild. In models of small vessel disease there is no injury directly induced in the brain, it occurs spontaneously. Published findings and our own experience indicates that any such brain damage is relatively minor compared to acute stroke and therefore has less of an effect on the animals, most being in the mild or moderate category. As animals age however effects can be more pronounced, though not in the severe category.

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

- Killed

#### **A retrospective assessment of these predicted harms will be due by 05 January 2028**

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

## 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?**

Studying mechanisms involved in brain diseases such as stroke and small vessel disease is extremely complex. Alongside the death of cells in the brains of stroke and small vessel disease patients, these conditions are characterised by changes in behaviour. Such behavioural changes cannot be studied in cell culture or other non-whole-animal alternatives, such as tissue slices or organoids. In addition, there are complex multi-system effects taking place in cerebrovascular disease, which are important in determining the outcome for patients. Such multi-system effects can only be studied in a whole living organism.

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

Non-animal alternatives are used wherever possible to address our aims, with the proposed animal studies complementary to a broad programme of work on stroke/small vessel disease using human samples, isolated cell systems and non-protected model organisms such as zebrafish embryos. The latter are used extensively in our programme of work on intracerebral haemorrhage and small vessel disease. This includes the ability to screen large numbers of drugs to find the most effective, before moving to studies in the mice and rats.

**Why were they not suitable?**

Non-animal alternatives are used wherever possible. However, these are not suitable to entirely replace the use of animals due to the complex nature of cerebrovascular disease and need to study processes under physiological conditions with all body systems intact.

**A retrospective assessment of replacement will be due by 05 January 2028**

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

## 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?**

Pathological and behavioural end points proposed in this project are well established from published studies of stroke and small vessel disease and experiments will be planned based on our own extensive experience alongside previously published data. We will use the minimum number of animals that can answer the desired scientific objectives and will extract all relevant information in the data by using appropriate statistical analyses.

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

Several factors lead to a reduction of animal numbers, including reducing variation (e.g. keeping the environment consistent), good experimental design (including the use of the NC3R's Experimental Design Assistant) and the use of appropriate statistics. Statistical tests will be used to ensure that we use the minimum number of animals possible to reliably interpret our data and so we can refine our questions to then design the most informative experiments. Whenever we get new data, we will always re-do our calculations to make sure we are still using an appropriate animal number to achieve our aims. We will also consult regularly with qualified statisticians about experimental design and statistical analyses.

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

To avoid unnecessary breeding, we will try wherever possible to obtain experimental animals from recognised suppliers and/or collaborators. Where in-house breeding is necessary, we will optimise the breeding programmes to obtain the required number of experimental animals as quickly as possible, using all offspring if we can. As soon as we have bred sufficient experimental animals the breeding will be stopped and animals maintained on minimum tick over, or the colony stopped if no further animals are required.

We will make optimal use of all animals e.g. harvesting multiple tissue samples for possible future use or for sharing with collaborators. For some work (e.g. in hypertensive animals) we will liaise closely with colleagues who will be able to use isolated blood vessels from the animals, leading to important additional information in addition to the biochemical/histological/functional data gathered in the primary experiment.

For many of our studies prior data is available for determining sample sizes. Where this is not the case, we will consult published literature and contact colleagues to see if appropriate data on variability and effect size can be obtained. In situations where such information is not accessible, we will perform pilot studies in small cohorts of animals. It is hard to be precise on exactly what number of animals will be used in such pilot studies, but we would typically expect  $n < 6$ .

We will make our data freely available to other groups through appropriate platforms so they can analyse it to answer their own research questions.



Critically, all our studies will be directly informed by the clinical situation, with back translation from observations in patients. This will be achieved through long standing and successful collaboration with clinical colleagues. We are also in the process of implementing a Patient, Carer, Public, Involvement and Engagement (PCPIE) strategy to ensure our research is relevant.

### **A retrospective assessment of reduction will be due by 05 January 2028**

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

## **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.**

We will use previously published methods to induce cerebrovascular disease, the choice of model being dependent on the hypothesis being tested. Models of stroke (both ischaemic and haemorrhagic) and small vessel disease are extremely well established in many laboratories across the world. Though there is no 'perfect' stroke or small vessel disease model, those to be used in this project are chosen based on their pathological and behavioural similarities to cerebrovascular disease in humans, which itself is extremely heterogeneous.

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

Our objectives cannot be fully achieved using less sentient animals (such as fish/insects) or with very young (neonate) animals due to differences in their nervous and immune systems, and the fact that cerebrovascular disease is largely associated with older age. We do make use of zebrafish embryos within our research on intracerebral haemorrhage and small vessel disease, for example to screen possible drugs before they are used in mice and rats.

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

All animals will be closely monitored for adverse effects and procedures put in place to minimise these, using the IMPROVE guidelines, and any other guidelines that are relevant.

Optimal post-operative pain management will be used, guided by advice from the NVS.

For all studies and at all times, animals will be handled appropriately by trained researchers (e.g. using tube handling for movement in and out of cages) and the use of suitable home cage enrichment.

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

Throughout the project we will continually review the literature and engage with colleagues/collaborators to learn of any new refinements to the protocols that could be implemented. In this respect the applicant is a co-author on the publication 'The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of in Vivo Experiments), published in the Journal of Cerebral Blood Flow and Metabolism, a leading peer-reviewed stroke journal. These guidelines draw on a wealth of experience in modelling stroke in rodents and were produced through an NC3Rs working group that included veterinary surgeons and other experts in animal welfare.

We will also consult other relevant literature, publications, and recommendations from the most appropriate bodies such as the NC3Rs and LASA, as well being informed from communication with the NVS and NIO and developments within the scientific community in general. For example, for refinements involving injections we refer to Morton et al 2001 and <https://researchanimaltraining.com/articles/an-introduction-to-the-administration-of-substances/>

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

We will always aim to implement any advances in techniques that adhere to the 3Rs and improve the welfare of the animals. We will stay up to date with the NC3Rs literature and recommendations, through the NC3Rs newsletter and communications with the Regional Programme Manager. Will also be informed from regular communication with the named veterinary surgeon (NVS), named information officer (NIO) and the scientific literature in general.

**A retrospective assessment of refinement will be due by 05 January 2028**

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?