



Home Office

## NON-TECHNICAL SUMMARY

# Applying Regenerative Neuroimmunology to Chronic Spinal Cord Injury Research

### 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

Neuroimmunology, Chronic spinal cord injury, Spinal Cord-Immune Interactions, Regenerative Medicine, Therapy

### Animal types

Mice

### Life stages

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?

The aim of this project is to identify and manipulate key mechanisms governing the interactions between the immune system and the spinal cord in the context of chronic spinal cord injury. The ultimate goal being to foster the intrinsic repair capabilities of the spinal cord in a way that halts the secondary damage associated with injury and stimulates mechanisms of neuroplasticity through the regeneration of the injured cord.

### **A retrospective assessment of these aims will be due by 20 April 2027**

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?

The United Kingdom experiences more than 1200 new patients with spinal cord injury (SCI) every year, the majority due to traumatic events with the remainder resulting from disease, such as the formation of non-cancerous tumours. Following SCI, the immune system is activated and results in the infiltration of immune cells into the damaged spinal cord tissue. Initially, the inflammatory immune cells are beneficial in the clean-up of cellular debris and damage, as well as forming a scar around the wound site to prevent the spread of inflammatory immune cells into the surrounding healthy tissue. However, the continuous presence of inflammatory immune cells damages the underlying nerves, meaning that messages travelling along the nerves become slowed, disrupted, or permanently halted. This causes a wide range of potential symptoms, including problems with bladder control, sensation, and partial or complete paralysis of arm and/or leg function that depends on the location in the spinal cord where the injury occurred (i.e., injuries to the spinal cord near the head effect the arms and legs whereas injuries near the waist only effect the legs).

The main function of the immune system is to protect its host against a wide range of pathogens, including bacteria, viruses, and parasites. However, there is an increased recognition that the immune system also plays a main role during brain and spinal cord development by controlling the generation of new nerve cells and that of the cells making the insulation of nerves (myelin), as well as that of new connections between nerve cells. Ultimately, cells and molecules of the immune system have a main function in prolonging inflammation in the spinal cord that contributes to the extensive tissue damage observed in SCI.

Neuroplasticity is the ability of networks of neural cells to adapt and change through (re)growth and reorganization, and it also implies protection from cell death, generation of new nerve cells and support cells, and changes in the amount of scar tissue.

Recent evidence has now shown that immune cells can be redirected towards a beneficial function, as seen in brain development, that could be harnessed to stimulate mechanisms of tissue plasticity and improve the clinical outcomes of patients with chronic SCI.

Previous work in the lab has in fact identified new ways to turn bad immune cells into good and promote the healing of the injured spinal cord in laboratory animals.

We have shown that the transplantation of a type of stem cells that is obtained from brain tissue into mice is able to interfere with the actions of the immune system in a way that lessen the damage in the persistently inflamed spinal cord. In addition, when brain-derived stem cells were transplanted into mice with SCI, these brain-derived cells could turn bad inflammation into good and promote the re-growth of damaged nerve fibres and reduce the amount of scar tissue. These experiments have clearly shown that modifying the interaction between the immune system and the spinal cord could be key in inducing neuroplasticity in chronic SCI.

With this license, we wish to further develop and explore novel ways to manipulate the interactions between the spinal cord and the immune system, to therapeutically manipulate inflammation and promote the regeneration of the damaged spinal cord. Amongst a number of potential mechanisms, we will focus on disentangling the way immune cells, which normally respond to bacteria and viruses, impact the spinal cord tissue after SCI. In fact, preliminary evidence suggests that altering immune cell activity can shift the cells and molecules found in the SCI from a toxic, cell death-promoting environment, to an environment that encourages regeneration. As such, this is a promising new approach to treat chronic SCIs. With this license, we will further investigate hypotheses such as these. Here, our ultimate goal will be to identify new and key molecular and cellular mechanisms of spinal cord-immune interactions that can be targeted with novel experimental molecular therapies. We envision these therapies will, ultimately, promote neuroplasticity in chronic SCIs through tissue regeneration in follow-up clinical trials.

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

The main output of this project will be new biological information on the function and therapeutic potential of mechanisms controlling persistent spinal cord inflammation. Additional outputs will include the publication of experimental data in scientific journals, and generation of accessible datasets. Further products of this project will be patents protecting the main discoveries, tools to address mechanisms or deliver therapeutic agents, and technologies to identify effects.

Ultimately, this project will translate into new therapeutic approaches capable of modifying inflammation in chronic SCI and promote neuroplasticity via modulation of spinal cord-immune interactions.

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

In the short term, the main beneficiaries of this project's outputs will be researchers from academic institutions and from industry. In the medium and long term, the outputs generated by this project will help the NHS and the patient community in the advancement of treatments for chronic SCI. These treatments will be aimed at preventing persistent inflammation of the spinal cord and promote neuroplasticity through the regeneration of the injured cord.

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

The outputs of this work will be disseminated to academic scientists throughout the duration of the license. We will communicate regularly with organized research networks and laboratory groups in the field of spinal cord injury research. These networks will allow us to share workloads and ideas, which depend on the expertise of each group, avoiding duplication of the experiments and accelerating the progress in these fields.

Preliminary data will be shared at national and international conferences and/or workshops in order to gain valuable feedback of the work from peers. This will also provide us the opportunity to build new collaborations locally and internationally to improve the quality and rigor of our research for the duration of the licence. We will also present our findings ahead of publication on preprint servers (such as arXiv, bioRxiv, or PeerJ).

When finalized, all our data will be published in peer-reviewed scientific journals. These data will include gene, protein, and metabolic throughput screen datasets, which we will also make available on appropriate databases for other research groups to access freely.

We are committed to regularly publishing both positive and negative results to increase awareness and inform the community of how our findings fit into the wider field and suggest which experimental outputs are worth progressing and which we feel would not be worth further exploration.

Finally, we will ensure that the published results are open access to maximise their impact and increase global awareness to both the public and fellow scientists.

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

- Mice: 1000

## **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.**

Up to five days old newborn mice that have been produced on a different licence will provide us with cells for use in modelling certain aspects of their responses and function in a lab dish.

Unfortunately, it is extremely difficult to accurately and fully model the complexity of SCI using cells in a dish. Therefore, we are using mice as they are widely used in pre-clinical 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, including formation of scar tissue, presence of inflammatory immune cells, and damaged of nerve fibres. In particular, the possibility to use genetically modified mice in which the expression of specific genes can be monitored and/or induced/reduced allows the study of target proteins and pathways involved in brain and spinal cord injuries and repair.

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

Mice will be fully anaesthetised and fixed in a device to maintain a stable body position. Then, a laminectomy will be performed, which is a surgical procedure where a small portion of the thin membrane covering the spinal cord is exposed to reveal the underlying thoracic tissue (i.e., the middle portion) of the spinal cord. From here, a computer-assisted impactor, which allows fine control of tissue damage and measurement of the actual impact force, will be placed over the exposed spinal cord tissue. The impactor then launches a weighted object that hits the exposed spinal cord tissue resulting in a contusion (i.e., bruising) SCI. Alternatively, some mice will receive a sham surgery (i.e., laminectomy only) but with no injury to the spinal cord tissue. This surgery is required to generate non-injured controls to allow for the comparison of cellular and tissue responses in the injured versus non-injured spinal cord. Following contusion SCI, mice will be left to recover until fully awake and responsive. The weight and overall body condition of the injured mice will then be monitored daily throughout the length of the study.

Contusion SCI mice may receive additional injections. These injections can be intravenous (e.g., into a vein), intraperitoneal (e.g., into the abdomen), subcutaneous (e.g., under the skin), intrathecal (e.g., into the fluid filled spaces of the spinal cord), intracerebroventricular (e.g., into the fluid filled spaces of the brain), intraparenchymal (e.g., into tissue), and local into the spinal cord. Injections will include either substances (such as drugs, beneficial small molecules, and agents to induce gene modification), viruses as a vehicle to artificially carry foreign genetic material into cells of the mice, and cells, which are all meant to foster the interactions between the immune system and the spinal cord in a way that results in a more robust regeneration, or less degeneration of the spinal cord.

Finally, to validate and identify biomarkers of SCI damage and their prospective value in the context of the regenerative process, we may also collect bodily fluids from live mice. These include blood and cerebrospinal fluid, which is a clear fluid that surrounds and cushions the brain and spinal cord from injury, for verification or discovery research using high-throughput screening technologies. Blood is obtained from a surface vein observable to the naked eye through a small prick using a fine needle, while cerebrospinal fluid is obtained via a quick, minimally invasive surgical procedure.

Experiments using contusion spinal cord injury may be as short as 1 day and/or may last up to 60 days (8 weeks or 2 months) post injury to study both immediate and delayed mechanisms of neuroplasticity.

The duration of each experiment is determined prior to the use of any mouse study and is variable in length. At the end of the experiment all mice will either be humanely killed, or tissues and organs collected under deep, terminal anaesthetic unconsciousness by first removing the blood by pumping a salt-containing liquid through the blood vessels, called perfusion, followed by tissue preservation in a fixative solution for follow-up analyses.

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

Within the contusion spinal cord injury protocol, mice display complete paralysis of both hind limbs initially but spontaneously recover within the first 2-3 weeks post-surgery and regain some degree of ankle movement. Mice are also expected to experience transient early pain for 24 hours after surgery along with some weight loss for one week. Since SCI affects the voluntary control of the bladder, mice are not able to express the bladder in full during the first days after surgery but will recover within one week. During this time, we will make sure the bladders are manually expressed by applying appropriate gentle abdominal pressure at least twice a day starting on the day after surgery, until mice recover the reflex (approximately 1 week).

In mice receiving additional injections, there could be transient pain and discomfort for a maximum of 48 hours (depending on the route of injection). Sampling of blood through the superficial tail vein will not lead to major adverse effects except transient discomfort for the animal. Sampling of cerebrospinal fluid may result in changes in food/liquid intake or in normal weight gain and/or transient (max 48 hours) local pain.

### **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)?**

Mouse: Moderate 10%

Mouse: Severe 90%

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

- ♦ Killed

#### **A retrospective assessment of these predicted harms will be due by 20 April 2027**

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?**

Spinal cord injury in humans leads to permanent and irreversible damage to the spinal cord resulting in lifelong clinical dysfunction, affecting the quality of life of patients and their carers. The development of therapies able to reduce the impact of persistent inflammation on the human spinal cord, while promoting its regeneration, is a main unmet clinical need for chronic SCIs. Since no experimental molecular therapy can be tested in humans without appropriate validation in relevant animal disease models, concepts developed using cells grown in a lab dish (i.e., cell culture) have to be tested and refined in animals where the complex environment of the adult nervous system is present and where functional recovery can be measured.

Over the course of the last two project licenses, our group has established a publication record and a pipeline of druggable pathways and molecules. We have also developed a strong understanding of models of inflammation and regeneration using immune and stem cells extracted from mice and stem cells generated directly from skin cells of humans using refined cell culture systems. Our functional and screening assays are based on the use of immune and brain stem cells, due to their role in controlling both the damaging as well as the regenerative responses in the spinal cord in the context of chronic SCIs. Indeed, under non-injured and non-diseased conditions in mice and humans, these cells help to maintain the optimal functioning of the brain and spinal cord. Hence, when the spinal cord is damaged, the response of these cells is important for re-establishing a normal function of the spinal cord.

To validate our cellular findings and new therapeutic treatments, we need to use rigorously validated and widely accepted pre-clinical mouse models of SCI. This allows us to test whether the immune responses we observe in cell culture and the effect of treatments are conserved effects within the very complex injury context. The immune response in injury is indeed very different in a mouse compared to a plastic dish. In a mouse, there are many physiological responses (e.g., inflammation, formation of scars, etc.) which directly impact the ability of the spinal cord to recover, as well as the efficiency of the treatments. Further, SCI also affects many other cell types present, such as nerve cells, which are challenging to study altogether in a dish. For these reasons, mice are widely used in pre-clinical research because their genetic, biological, and behavioural characteristics closely resemble those of humans, thus allowing the study of many of the changes seen in patients, including tissue scar formation, presence of inflammatory immune cells, and damaged nerve fibres. In addition, the possibility to use genetically modified mice in which the expression of specific genes can be monitored and/or altered allows the study of target proteins and pathways involved in central nervous system (CNS) injuries and repair.

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

Over the years our team has refined and improved upon our models of cells grown in lab culture dishes that allow us to test the tolerance and efficiency of our treatments before testing them in a mouse. Additionally, we have developed a new model of maintaining and expanding human immune and human stem cells in plastic dishes that does not involve the use of mice. This new system allows us to (i) capture the response of human cells in a dish and (ii) design/perform experiments to test ideas about

the response of cells without having to extract these cells from mice beforehand. After the identification of targets, we will then proceed to test our results in our mouse models of disease or lesion.

As an additional replacement strategy, we will be applying data mining. This is the process of uncovering patterns and other valuable information from large data sets. The creation of next generation technologies allows us to analyse the responses and behaviours of individual cells at an extremely detailed level. These analyses are being carried out by several groups across the world, including my own team. In fact, large datasets are made accessible to the public even before publication. These public datasets are stored on dedicated websites (i.e., biorepositories) or on websites where the manuscript is stored prior to review by other scientists (i.e., pre-preprint servers). Everyone can then access these public data and perform their own analysis for targets that are important for experimental studies. Data mining has improved upon organisational decision-making through analysis of these freely available data sets. The data mining techniques that will be used in these analyses can be divided into two main purposes. One, they can either describe the target dataset. Two, they can predict outcomes through the use of machine learning algorithms. Here, a small list of targets can be created to test hypotheses without having to perform the same set of experiments, including animals, that these large studies have already completed. This way, the need for the repeated collection of cells from animals can be extensively bypassed.

### **Why were they not suitable?**

Immune and stem cells grown in lab culture dishes are useful for studying some aspects of SCI, however they cannot replicate the complex changes that occur in the cells and tissues that support the function of the spinal cord. These includes changes to the supporting cells of the spinal cord (called glia, from the Greek for 'glue') that can lead to the formation of scar tissue, the recruitment of immune cells into the injury site, and damage to nerve fibres that is typical of chronic SCI. Immune and stem cells grown in culture also behave differently to those found in a living organism, showing loss of cellular heterogeneity (i.e., the unique identity of individual cells) and loss of communication with other cell types.

It is therefore necessary to use animal models to assess the complexity of biological and behavioural responses in an animal, both following an injury and after therapeutic treatment. There is also a requirement to demonstrate that a treatment is safe and effective in animal models before progressing to human application.

### **A retrospective assessment of replacement will be due by 20 April 2027**

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?**

Animal numbers were estimated based on a previous project licence and a combination of the retrospective review, annual return of procedures, and estimated animal usage for the duration of the project. With the new mouse colony management system (MCMS) in use, we are now afforded unparalleled access to total animal usage year-over-year. Our review of the 2020 data showed that a total of 13 mice were used for SCI studies. These data were severely affected by the current COVID-19 pandemic, therefore in the next 5 years we are expecting at least a significant increase in the SCI numbers per year leading to approximately 200 mice/year for SCI experimental studies for a maximum total of 1000 mice.

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

The National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) experimental design assistant is a tool which we constantly use to help the design and further refine our experiments.

According to our lab standard operating procedures (SOPs), experiments are constantly assessed at the pilot stage first (i.e., a first experiment is conducted with a reduced number of animals to adjust some key parameters before running the full experiment). This ensures that the correct number of mice necessary to achieve robust statistical results is used only when experiments are ready to be conducted in full. Mice are then placed in the experimental groups randomly, which helps to ensure case and control groups are homogenous. Treatments are given 'blind', which means that either the operator injecting a treatment (or a vehicle, as control) or the surgeon performing the injections have been given no access to the information related to the treatment they are giving. Blinding is also applied to post-mortem tissue and molecular studies. Unblinding is the responsibility of the principal investigator and it is done only after the experiment is concluded and results are analysed, to avoid any bias in the generation of the results.

**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 always perform pilot studies before undertaking a full experiment to ensure that larger studies are as accurate as possible. These pilot studies allow us to assess the experimental design and identify potential problems, as well as implement improvements early on in the licence. We are also coordinating with other groups to share animal tissues - including tissues from genetically modified mouse lines and post-mortem tissues - in order to further reduce overall mouse numbers.

**A retrospective assessment of reduction will be due by 20 April 2027**

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.**

Mice that undergo contusion SCI temporarily suffer and are in pain. However, the contusion SCI mouse model is the most refined for the purpose to cause the least pain, suffering, and lasting harm to the mouse and is a widely- and consistently used mouse model of SCI that has undergone continual refinements over decades of research.

The computer-assisted impactor used to induce injury provides calibrated and reliable readouts and allows fine control of tissue displacement and measurement of the actual impact force. This will allow us the flexibility to further refine our model to induce the minimum injury necessary to reach the experimental outcomes.

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

We are extremely limited in the use of invertebrates (e.g., worms), fish, or amphibia (e.g., frogs) as they are not fully suitable for the development and testing of clinically translatable new treatments for humans with chronic SCIs. In fact, while some preliminary work on regenerative biology is done in non-mammalian species, the complexity of the interactions between the immune system and the spinal cord in the context of tissue damage and regeneration can only be studied in mammals, as they possess striking anatomical and immunological similarities with the human spinal cord and immune system.

We also cannot solely rely on mice that have been terminally anaesthetized, as we need to assess the long term behavioural and pathological outcomes of our manipulations and interventions in promoting regeneration of the damaged spinal cord. Therefore, we need the mice to remain alive for several weeks-to-months after the onset of disease.

Finally, we need mice with cells that have reached a mature stage of development as representative of the cellular make-up present in adult human SCI. Therefore, we will use adult mice for the pre-clinical mouse model of SCI. The use of mice during the immature stages of life will be restricted to the collection of cells for cell culture studies using lab dishes where indicated and appropriate.

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

Before starting any study plan, we will discuss all experimental methods with the appropriate staff within the animal unit to ensure that all the necessary equipment is in place and that we are able to perform procedures under optimal conditions and/or supervision for the best health and welfare of the animals. Prior to running studies, we will determine if the necessary staff and expertise is available to successfully run the whole study, which skills are outstanding for the success of the study, and which relevant equipment is available to process samples under optimal conditions.

Once the study has started, we will rely on our established step-by-step care packages to ensure that the harm to the mice is as minimal as it can be, and the welfare of the mice is never compromised.

Over the years we have made significant refinements to our contusion SCI mouse model in order to minimize the pain, suffering, and distress of the mice. These efforts have culminated in a dedicated and comprehensive standard operating procedure (SOP) that provides a detailed step-by-step care package. Refinements to this model are centred around the post-surgical care and housing of injured mice and the daily care and monitoring of the mice.

Several refinements were made to the housing of injured mice. For example, we provide bedding that does not inhibit the free movement of injured mice and does not get caught in the surgical clips used to keep the surgical wound closed.

We keep the mice in heated chambers for at least the first night post-surgery, and then attach heating pads to the outside bottom of the cages to maintain stable core body temperature.

We also provide unrestricted access to wet mashed food and supplemental edible hydrating substances to encourage eating and hydration, respectively.

For the daily care and monitoring of the mice, we have increased the number of daily checks to ensure the health and welfare of the mice is maintained.

We have incorporated the use of pain medications immediately pre-surgery and then as needed for at least 24 hours post-surgery to ease disease complications.

We perform fluid replacement through subcutaneous (i.e., under the skin) injections if signs of dehydration are present. After SCI, mice lose their spontaneous urination reflex. Thus, to refine this harm we manually express the bladder by applying appropriate gentle abdominal pressure at least twice a day starting on the day after surgery, until mice recover the reflex (approximately 1 week).

Having a dedicated SOP ensures that the highest quality of care is provided to mice throughout the course of this study and aims to significantly minimize the suffering and improve the welfare of paralysed mice. This SOP has been generated and further refined following recommendations from animal technicians, named animal care and welfare officers, and the named veterinary surgeon.

In the case of surgery, we have put in place post-operative assessment sheets specific to our model of injury to help the technicians to monitor better the recovery of the mice after surgery. This post-operative assessment sheet is constantly refined during our work, depending on our observations and in collaboration with the animal unit staff. Incidence of pain during the post-surgical recovery period will be controlled by the administration of pain killers, as directed by the named veterinary surgeon. Since surgeries will inevitably cause transient pain, which resolves by 48 hours, this will be minimized by the

use of pain medications. Adult mice undergoing surgical procedures will rarely have complications 48 hours post-surgery. Regular discussion with a named veterinary surgeon will allow us to improve the management of pain if any new and more suitable recommendations appear during the work. In the case mice suffer from complications after surgery, appropriate guidelines are in place for humane endpoints.

Full training will be provided to new technicians who are unfamiliar with these procedures, as we have filmed previous study procedures to show how we expect our mice to recover. This helps new technicians to learn how to assess our mice correctly and in turn, means that mice recovering in our experiments receive the same high quality and consistent level of monitoring and care they need.

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

We plan our experiments in accordance with the guidance provided in the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines. This will guarantee we use the minimal number of animals to answer our objectives and ensure our results are both robust and reproducible. We will follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines when preparing our data for publication. In so doing, we will ensure our published findings are complete and clearly presented and easily accessible to other groups. This will lead to a reduction in the unnecessary duplication of animal experiments.

Excellent information is available on our establishment website, which is routinely updated with new 3Rs information. The National Centre for the 3Rs (NC3Rs) website will be regularly consulted to be sure that we are applying the latest recommendations for the refinement of our experiments. The Laboratory Animal Science Association (LASA) website provides updated information, especially regarding best research practices to perform aseptic (i.e., germ free environment) surgeries. We will also consider any new publications in a peer-reviewed journal relevant to our field offers refinements to our protocols.

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

Our establishment offers continuous training and recommendations via the animal facility and from animal care staff located within. We will keep informed of any changes to animal welfare guidelines by regularly consulting the website they provide to ensure that maintain compliance should any new updates be posted.

The National Centre for the 3Rs (NC3Rs) will be the main reference to assess whether our experiments match the highest standards of 3Rs, and we will adapt our protocols if the recommendations evolve throughout the duration of this project. Regular consultations on the latest practical guidance from Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) will provide additional sources of new recommendations and advances in animal techniques and clinically applicable models.

Training records for all personal licence holders will be kept up to date using a centralized database. Senior group members will provide extensive training on the relevant regulated surgical or non-surgical

procedures to all new lab members who will be working with mice as part of the ongoing project. Further, new lab members will be informed of the (optional) training services available to them. This will guarantee that general practices are firmly adhered to, which will ensure the welfare of the mice is consistently applied.

As a licence holder, it is my own responsibility to stay updated on published best practices by consulting information for licence-holders provided by our establishment and by speaking to other project licence holders.

**A retrospective assessment of refinement will be due by 20 April 2027**

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?