Home Office

NON-TECHNICAL SUMMARY

Role of steroid-producing immune cells in inflammation, immunity and cancer

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

steroids, immune regulation, cancer, inflammation, immunotherapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

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?

Recently, we discovered that immune cells can produce steroid hormones (Steroid hormones: A group of biological chemicals mainly secreted by “steroid glands” such as adrenal glands, testes, and ovaries). This project aims to discover the basic principles of immune cell-mediated steroid hormone production, and their role in regulating infection, inflammation, and cancer, as well as to exploit this newly created knowledge to develop new therapeutic approaches.

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?

Immune cells are required to fight infections by bacteria and viruses. However, their disordered or dysregulated function causes many disease processes. When immune cells attack the own body, it is called autoimmunity. In allergy, immune cells respond to nonharmful substances and show pathology. When immune cells fail to recognise and kill cancer cells, it leads to tumour progression. The immune system comprises diverse cell types that can promote or inhibit inflammation and immune responses as required. Suppressive or inhibitory components of the immune system are required to restrict excessive or uncontrolled immune reactions. However, if maladapted, they can suppress potentially beneficial immune responses. Such maladaptation is known as immunosuppression (Immunosuppression: Suppression of the immune system). Immune reaction and immune suppression are targets for the development of new drugs.

Newly developed therapies targeting mechanisms that suppress immune cell function (i.e., immunosuppression) have shown promise in cancer patients. These drugs unleash the power of immune cells and help kill cancer cells. However, the knowledge of immunosuppression mechanisms is limited. In this study, we will create new knowledge by revealing the basic principles of one such immunosuppression mechanism. We discovered that certain types of immune cells produce steroid hormones. These steroid-producing cells suppress immune responses. In cancer, they suppress anti-tumour immune responses. Steroid hormones are a group of biological chemicals mainly secreted by “steroid glands” such as adrenal glands, testes, and ovaries. Steroid hormones regulate immune cell function. In general, they are immunosuppressive and anti-inflammatory. We will investigate how immune cell-produced steroids regulate cancer, inflammation, and immunity. The study should lead us to discover new therapeutic targets and therapeutic agents against cancer. Modulation of these steroid-producing cells would benefit certain types of cancer treatment and inflammatory diseases like allergies and asthma.

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

On a global scale, a common practice in the clinic is to target immune cell function as a therapeutic approach to bring health benefits. For example, therapies targeting immunosuppressive mechanisms in cancer are presently revolutionising the treatment for patients with metastatic disease. This type of approach unleashes the power of the body's immunity against cancer. By contrast, anti-inflammatory and immunosuppressive drugs like steroids are used to treat allergic asthma, allergies, and auto-immunity.

This study will extend our basic knowledge of how the immune cells' function is controlled or regulated. In particular, we will learn how steroid-producing immune cells and their secreted steroids regulate immune cell function during inflammation and immune reactions, including cancer. We will identify and confirm new therapeutic targets for inflammatory and immune dysfunction-related diseases such as cancer, allergies and asthma. We will publish our research in peer-reviewed journals and present our findings as oral presentations and posters at national and international conferences. The research will also provide a basis for the development of new therapies aimed at controlling immune function in patients with a variety of disorders in which the immune system plays a critical role in inflammatory diseases, infection, and cancer. More specifically, we will demonstrate that intervening immune cell steroid signalling may bring benefits for cancer-associated inflammation and immunity. At the same time, we will show evidence that augmenting immune cell steroid signalling may bring benefits for inflammation and allergic asthma-related disorders. We will develop new therapeutic immune cells and test their efficacy in mice models. These therapeutic cells could be used in treating cancer and inflammatory diseases. These novel products with therapeutic potential will be patented.

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

The project will benefit researchers in related scientific fields, such as cancer immunology, inflammatory disease biology and infectious diseases. The work is relevant to scientists aiming to develop new therapies for individuals with immune-mediated disorders, cancer, and infection, including pre-clinical researchers and the pharmaceutical industry. The impact of these diseases and disorders on human life is awfully daunting. According to the World Health Organisation (WHO), **cancer is a leading cause of death worldwide**, accounting for nearly 10 million deaths in 2020, only. The most common in 2020 (in terms of new cases of cancer) were: breast (2.26 million cases); lung (2.21 million cases); colon and rectum (1.93 million cases); prostate (1.41 million cases); skin (non-melanoma) (1.20 million cases); and stomach (1.09 million cases). The most common causes of cancer death in 2020 were: lung (1.80 million deaths); colon and rectum (916 000 deaths); liver (830 000 deaths); stomach (769 000 deaths); and breast (685 000 deaths). Each year, approximately 400,000 children develop cancer. The most common cancers vary between countries. Our research suggests that steroid-producing immune cells are involved with all types of solid tumours tested, however, not all patients show this mechanism of immunosuppression. It warrants further studies. In the future these patients will be benefitted. No statistics are available on how many researchers are involved; however, the number must be big.

WHO reported that **Asthma** affected an estimated 262 million people in 2019 and caused 455,000 deaths. Other **inflammatory diseases**, such as allergies, are a big burden to healthcare and a major topic in immunology and medical research. This project is expected to bring benefits to these patients in the future.

Downloaded: 11:23am, 6 May 2025

As **this project will identify and confirm therapeutic targets of cancers and inflammatory diseases (such as colitis, colitis-driven cancer, allergies and allergic asthma)** and virus infection, in the long term, the knowledge may bring patients benefits from the above-mentioned diseases. To expedite and amplify this possibility, we will immediately share the research results with the community. Whoever is involved with the research on these areas will benefit.

In addition, the intellectual property generated by this study might be commercialised in the future.

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

The outputs of this project will be disseminated through research publications and presentations. Data and reagents will be directly shared with academic and industrial sector researchers ahead of publication. We collaborate with multiple research groups of the field. We also strive to engage the public with our science. We will secure intellectual property and commercialise our research to foster UK industrial and bioscience growth.

The unsuccessful approaches will be submitted and pre-printed on bioRxiv (https://www.biorxiv.org/), an online archive and distribution service, for sharing with global scientists. We will also communicate these results for publication in peer-reviewed journals.

Species and numbers of animals expected to be used

- Mice: 28,330

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.

In vitro (in a test tube, culture dish, or elsewhere outside a living organism) reproduction (modelling) of the immune reactions (interactions between immune cells and with the inflamed tissue) is extremely difficult. It often does not reproduce what happens in a living organism. This is because of the complexity of the immune system. Immune responses are dynamic interactions between multiple cell types and tissue types. In other words, immune response is an integrated physiologic process. The immune system in mice is similar enough to the immune system in humans. Many genetically modified mice and reagents are available to study the immune system. Therefore, this species can be used more efficiently than any other species to study the role of genes that regulate the immune system.

In general, we usually use adult mice. The immune system of adult mice is similar to that of humans in many ways. Our research focuses on diseases which primarily affect adults. A similar is true for the endocrine system. The endocrine system is made up of glands that make hormones. Hormones are the body's chemical messengers. They carry information and instructions from one set of cells to another. The endocrine system influences almost every cell (including immune cells) of our body. In

Downloaded: 11:23am, 6 May 2025

adults, the endocrine system is stable and well-developed. We will use mice of similar age to reduce the variability of our experiments. Apart from studying adult mice, we will study aged mice in a few experiments to investigate the effect of ageing. We discovered this new fact: Immune cells can produce steroid hormones. Mice, adult and aged, would help us understand the role of these steroid-producing immune cells.

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

Most of the mice will be used in breeding genetically modified animals. We will use two types of genetically modified mice. Most of the genetic modifications are in the immune cells (that regulate immune cell function). Some of the mice would have genetic mutations that cause cancer development. These genetic modifications will either help identify those cells or be used to test the effect on immune cells' regulation. These genetically modified mice will be used in experiments. All the mice will be killed in a humane way, and the organs related to immune cell function will be analysed in the laboratory. These mice will be used in experiments to test how the immune system responds to infections, inflammation, and cancer. Typically, animals will experience mild, transient pain and no lasting harm from the administration of substances by injection using standard routes (into the tail vein, under the skin, into the peritoneal cavity).

Most of the experimental mice will be used to test the immune response against tumours. We will implant small numbers of tumour cells under the skin (i.e., subcutaneous injection) of animals and let them grow. To test the effect of a gene, we will use mice with genetic alterations in the immune system to tumours. Tumour growth will be monitored regularly by trained staff. Animals will not be allowed to suffer excessively, or beyond well-defined criteria. Animals that are likely to develop adverse effects that extend beyond the well-defined limits will be killed using a humane method before they reach that point. After humanely killing animals, tumours and organs, such as spleens, lymph nodes and lungs, will be taken and analysed in the laboratory to gain insights into how immune cells respond tumours. The conclusion of the experiment will be how a specific gene controls immunity against tumour. In general, these experiments take around three to four weeks from tumour injection to humane killing. Animals may receive substances (known drugs or potential drugs) during tumour growth to examine the therapeutic potential. Similarly, we will test animals' immune responses against experimental diseases (infections and inflammations). The typical duration of such disease models is up to 16 weeks.

We also need to use models of inflammation-induced cancer, infection, asthma and allergic inflammation to test the role of steroid-producing immune cells, in a very limited number of experiments. In a few experiments, experimental mice will have received therapeutic immune cells derived from another mouse or immune cells with known effector function generated outside of the body (in sterile Petri dishes or flasks). In such experiments, animals will typically receive an injection of immune cells in the tail vein and then infection or inflammation or cancer will be induced. To induce inflammation or allergy or cancers, well-established substances/agents will be administered via any one of the appropriate routes: via nose inhalation, via mouth with food or drinking water, into or under the skin by injection, into the tail vein, into the peritoneal cavity, and rectal route.

Animals will be routinely assessed for signs of illness and weight loss. Animals likely to breach the severity limits of the protocol will be killed humanely.

Downloaded: 11:23am, 6 May 2025

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

Most of the animals will be used in breeding to expand genetically altered mice, which is essential for the research and will experience no significant adverse effects. Adverse effects from experimental procedures depend on what experimental model is being used. For example, likely adverse effects in cancer models include the ulceration of tumours and skin damage over the site of injected tumour cells. Ulceration triggers a decision to the humane killing of the animal. Mice may show breathing problems in the lung tumour model. Mice with signs of laboured breathing will be humanely killed. Mice may lose weight. Mice with 15% weight loss will be humanely killed. Experimental animals in the colitis model may lose weight and exhibit reduced activity. Animals with colitis are expected to have diarrhoea. Blood is occasionally observed in the stool. Mice will be humanely killed if their weight loss reaches 15%, or if they show clear signs of diarrhoea and rectal bleeding that does not resolve within 24 hours. Each cycle of the colitis model (in the chronic colitis model) persists for 3 to 5 days with a minimum gap of two weeks that helps them recover. Administration of influenza virus, on a maximum of two occasions at least two weeks apart, may cause body weight loss but does not prevent normal feeding, drinking, or other normal activities. Colitis induces tumour formation. In colitis-driven tumour models, administering colitis and tumour-inducing agents is expected to result in intestinal inflammation and weight loss. Any mice that reach 15% weight loss will be humanely killed. In the asthma model, the mice are expected to have trouble breathing. In the skin inflammation/allergy model, localised skin itching, discomfort, mild pain, and skin scratching and picking (localised at inflamed area) behaviour are expected. However, reactions are expected to be highly localised to the area of topical administration and the induction of a whole-body symptoms is not expected. Skin inflammation (atopic dermatitis) model typically takes up to 1 month and involves repeated (no greater than 10) topical administrations of a chemical that induces skin inflammation (dermatitis), usually on both surfaces of the pinna of one ear. Animals will receive substances, such as antibodies and steroid hormones, prior to or during the induction of inflammation. During or after the resolution of inflammation, animals will be humanely killed, and their tissues will be analysed in the laboratory.

A limited number of NSG mice (NOD scid gamma mice) will be used. These mice are deficient in many of the immune cell types. An estimated 20% (or less) of mice from these NSG mice may 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.

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 80%

moderate 20%

What will happen to animals used in 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?

Modelling human diseases and related immune reactions in animal models is necessary to understand how our immune system works. Several crucial interactions of immune cells with other cells and substances in living animals cannot yet be generated (modelled or reproduced) in test tubes or cell culture dishes. Immune cells are distributed throughout the body (in different organs). They are able to migrate into almost all body tissues to protect the body from infection and cancer. These features can not be reproduced artificially. Therefore it is essential to investigate immune cell behaviour and function in the whole animal. Adaptive immunity-related immune cell function (the type of immunity which remembers previous exposures of antigens, for example, how a vaccine works), which is the subject of this research, evolved in vertebrate animals and is not present in less sentient organisms. Many cell and biological molecule-related features among vertebrates are highly conserved between mice and humans. Many useful tools and well-established models for experiments in mice already exist, allowing us to perform research using mice in an efficient manner that minimises the number of animals we need to use. Therefore, the use of mice in this research is necessary.

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

We considered computer-based (computational) analyses. These coputer-based approaches (i.e., bioinformatics and cheminformatics approaches) use publicly available data and predict molecular and cellular interactions. We also considered cell cultured-based experiments that are done in Petri dishes and test tubes for certain molecular pathways that have an important role in controlling the immune system. For example, undertaking a computational approach of drug screening and drug repurposing study, we identified novel drugs that are already approved but can be repurposed for cancer treatment. These drugs modulate the immune cell-mediated steroid production and action of immune cell-produced steroids. To test the efficacy of those drugs, some cell culture-based experiments were sufficient to make a conclusion for our biological questions. However, many key questions (the most important questions) remain unanswered from such experimental approaches. For those animal experiments are essential. For example, whether predicted cell-to-cell and molecular interactions are really happening inside the body of an animal. We used steroid-producing cell lines and laboratory-generated steroid-producing cells. We use human patients' samples on a regular basis to check the presence of steroids within the tissue, gene expressions that are involved with steroid production and steroid action, and proteins that are involved with steroid-mediated cancer progression. We use human blood obtained from healthy donors and patients to test the role of immune cell-mediated steroid production and the effect of those steroids on human immune cell functions. To fulfil the same purpose, we also use several cancer and immune cell lines. We engineer human blood-derived immune cells to generate genetically engineered versions that can be used as therapeutic cells in the future. Altogether, these alternative approaches replaced at least double the number of mice needed to test directly in mice models.

However, there are unanswered questions related to how the immune system works within the body and for what mice work is most suitable but with the least suffering and pain.

Why were they not suitable?

Computational analyses, test tubes or cell culture-based experiments using purified immune cells fail to reveal the various interactions of immune cells with other cells and substances in the body. Moreover, organs and tissues contain complex mixtures of both immune and non-immune cells, each of which can signal to other immune cells and affect their behaviour. Reproducing such a living tissue set-up is impossible at present. Insights into whether specific components of the immune system can be targeted to improve therapeutic outcomes are also difficult to gain using experiments performed in test tubes or culture dishes. We will perform two types of animal experiments that are impossible to model in the culture dishes or test tubes. First, the role of steroid-producing immune cells in different animal models of diseases (mainly cancer, but also in asthma, allergic inflammations, and colitis). In particular, we will validate (confirm) the predictions that are derived from computational analysis and test tube or cell culture-based studies. Second, we will modulate (induce or reduce) the immune cell-mediated steroid production and steroid action, genetically or pharmacologically, to test whether such modulation can be used as a therapeutic strategy. Before arranging clinical trials in human patients, such a preclinical experimental approach in a limited number of mice would be necessary to demonstrate the proof of the principle. Reproduction of such pathological situations and disease outcomes because of genetic and pharmacologic targeting of immune cell steroid production is impossible in test tube or cell culture-based studies.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have provided the estimated number of mice to be used under each protocol of the license. The use of littermate controls is a requirement in our experiments to ensure an identical environmental and genetic background between experimental and control animals in experiments. We calculated the number of mice required per line to produce sufficient experimental and control animals from heterozygous (consisting of different alleles of particular genes) crosses to allow for experiments to be conducted with adequate statistical power. We used statistical tools that are designed to help researchers find the least number of animals. The calculated numbers are sufficient for the generation of genotyped control and experimental animals at a typical age (range 8-12 weeks) at which they can be used in experiments. Our calculations are based on average litter sizes of 6, an average time between litters of 6 weeks, an expected sex ratio of 0.5 and an expected Mendelian ratio of 0.25 for experimental and control progeny. All experimental mice will be a continued used from the protocol 4 (i.e., breeding and maintenance of genetically altered mice).

Downloaded: 11:23am, 6 May 2025

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

Experimental control groups are important for most of the experimental models being used and provide a means to improve data quality from our experiments involving animals. The generation of high quality data eventually provides a means of reduction since definitive results will prevent the necessity to perform experiments again, either due to technical failure or a lack of broad applicability/interpretability to other researchers in the field. We use resources available for designing experiments with an appropriate randomisation and blinding strategy and sufficient sample sizes to enable adequate statistical power, such as the NC3Rs experimental design assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda). Many similar experiments have been done before in collaboration with our collaborators. We used those experiments and cohort size as benchmarks. We use online available experimental designing tools, which are designed to minimise the number of animals used in experiments but sufficient to draw conclusions from 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 monitor the breeding performance of our mice to ensure that the minimum numbers of animals are used. We use colony management software that helps optimum production and avoid overproduction. The use of inbred strains helps to model experiments with definitive conclusions using minimally required mice. The strategy minimizes variation and allows us to robustly ascribe the cause of phenotypes observed to the introduced genetic mutation. Our research will require the generation and maintenance of complex multiallelic mouse genetic strains at colony sizes sufficient to provide littermate experimental and control animals from heterozygous mating. Our calculations were based on previous experience generating and maintaining multiallelic mouse strains obtained from our collaborators who perform similar experiments.

The maintenance of specific pathogen-free health status and controlled environment will reduce experimental variability and decrease the cohort size required for sufficiently powered statistical analysis. Members of the team receive training in statistics used in our experiments. Biological plausibility, mechanistic insight and consequences of the effect will inform the interpretation of significance in addition to obtained statistical significance values. Studies will be reported according to ARRIVE guidelines.

We keep ourselves up to date with the literature for methods to reduce the numbers of animals required and to improve experimental design. If a suitable allele has been generated elsewhere, we import this by embryo or sperm transfer, or by shipping live mice where sperm or embryos are unavailable, and inter-crossing the offspring to obtain the required genotype. Cryopreservation and re-derivation by embryo transfer are important for both the quality of the science and for a reduction in the number of animals bred.

Genome editing technologies such as CRISPR/Cas9 mediated gene editing offer a potential for reduction. CRISPR/Cas9 allows the function of genes to be tested without germline mutagenesis and the establishment of mouse colonies to test gene function. We have started to develop powerful new high-throughput CRISPR/Cas9 screening approaches to identify functionally relevant genes within specific immune cell types in vitro. While not relevant to a substantial proportion of our present

Downloaded: 11:23am, 6 May 2025

research, this approach has the potential to lessen our need to perform preliminary testing of gene function using germline mutagenesis with subsequent reduction in mouse usage.

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 minimise suffering by carefully observing the mice undergoing procedures and adhering to AWERB handbook guidelines. Following the guidelines, we will assess clinical signs that may trigger the end of an experiment for a particular mouse or a cohort. We will choose the model which causes the least suffering to animals but is sufficient to answer a specific question.

We prioritize using mice that have become singly housed, as soon as possible. In consultation with animal technicians and veterinary staff, we modify protocols and introduce refinements to the protocols and animal husbandry that reduce harm. This includes alternative bedding for animals with reduced mobility, providing access to food in gel format, use of analgesics and more frequent monitoring for mice at increased risk.

Where tumour immune responses are being tested, we will primarily implant tumours by subcutaneous injection. This model minimises animal suffering by enabling non-surgical introduction by needle injection of tumour cells into animals. Further, the subcutaneous space provides a compliant space for tumour growth resulting in minimal suffering to mice as tumours develop. In general, subcutaneous or intraperitoneal injection of tumour cells is preferable from an animal welfare perspective to surgically implanting cells into the internal organs in recipient animals. Therefore, subcutaneous tumour models form the mainstay of the proposed work involving tumour immunity, although non-surgically implantable locations will also be used, such as melanoma tumours injected into the intradermal space, and breast carcinoma cells injected into the mammary fat pad, ovarian cancer cells into the peritoneal cavity.

Infection with the influenza virus will be carefully controlled to minimise adverse effects by using the least harmful strain and minimum effective dosage (as reported by previous studies). Batches of infectious agents will be standardised using in vitro assays, and the lowest dose will be sufficient to elicit the required immune response given. Similar strategies have been taken for all inflammation-related protocols where the choice of inflammatory agents and their dosages are kept that is minimally required. Additionally, we have established clear humane endpoints for all protocols to avoid unnecessary suffering of infected animals.

Perioperative and post-operative analgesia will be given when necessary, using advice from the NACWO and NVS. The choice of analgesic, duration and dose will be adjusted to the clinical signs observed considering possible impacts on the experimental plan. Perioperative analgesia will be given and maintained after surgery for as long as is necessary to alleviate pain. Analgesia will also be

Downloaded: 11:23am, 6 May 2025

administered to control pain for painful procedures. In addition, procedures are refined to reduce the suffering they cause and training of staff conducting procedures is maintained to minimise inter-operator variation and suffering.

Administration of tamoxifen in the diet is known to cause neophobic effects in mice. We have gained experience from our collaborators and information from our project support team that has enabled us to make refinements to our tamoxifen administration protocol reducing adverse events related to tamoxifen-induced neophobia substantially.

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.

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

Lesser model organisms, such as zebrafish, have far less conserved immune systems to humans than mice, are less well characterised and many of the tools used to study immune function in mice have not been generated for lesser species. Thus, while we have considered other ways to reduce and refine our use of animal models, our funded research programme would be impossible to complete using less sentient species than mice. Access to primary and secondary lymphoid organs and peripheral tissues, such as the lungs, gut, spleen and lymph nodes, is required for the proposed analyses. Such access is limited in human studies. Adoptive transfer experiments are also possible using inbred mouse strains since they are genetically identical and do not express mismatched antigens for graft rejection.

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

We constantly refine our experimental techniques to prevent unexpected adverse events, minimise variability and thereby reduce experimental group sizes, and minimise suffering caused to animals by working with small groups of animal technicians longitudinally on specific projects so that experience of experimental techniques builds over time, and by producing and refining standard operating procedures for interventions such as cell injection and tumour measurement.

We monitor animals daily. We will keep close contact with animal technicians to increase the monitoring frequency and measurements when necessary and humanely kill animals once any significant clinical signs show. We will continue to enhance this practice.

To reduce the pain caused by administering any immunoregulatory substances, we will seek advice from animal technicians, NACWOs and NVSs.

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

We will follow these guidelines below:

Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy.

Downloaded: 11:23am, 6 May 2025

Lab Animal 46(4):152-156. doi:10.1038/laban.1217

LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.).

Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T (2018)

PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines

ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines

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

1. We will have regular discussions with the researchers, animal facility technicians, and managers at our institution to review current approaches and whether there are any new 3Rs.

2. Research staff will subscribe to the NC3Rs e-newsletter, providing updates on updates to 3Rs principles and methods, funding opportunities, 3Rs events and publications.

3. We will encourage staff to attend NC3Rs events and workshops as a way of keeping abreast of 3Rs advances and approaches.

4. We will contact NVSs (Named Veterinary Surgeons), NACWOs (Named Animal Care and Welfare Officers) and animal technicians to seek advice when necessary.

Downloaded: 11:23am, 6 May 2025