Home Office

NON-TECHNICAL SUMMARY

Molecular mechanisms of tolerance and immunosuppression

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

cancer, immunosuppression, immunological tolerance, infection, inflammation

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

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?

Our research aims to discover new mechanisms underlying immunological tolerance and immunosuppression, and to define mechanisms that have distinct rather than shared roles in these processes.

A retrospective assessment of these aims will be due by 20 October 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 its 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 normal function of the immune system is required to fight infections by bacteria and viruses while its disordered function contributes to many disease processes including autoimmunity, allergy, chronic infection and cancer. The immune system is composed of diverse cell types that can either promote or inhibit immune activation. While inhibitory components of the immune system are required to suppress autoimmune and allergic inflammation in a process referred to as immunological tolerance, they can also suppress potentially beneficial responses against infections and cancer, in a process known as immunosuppression. Both are important immunological processes and targets for the development of new drugs.

Newly developed therapies targeting mechanisms of immunosuppression have shown promise in activating immune function in patients with cancer, but since the mechanisms of immunosuppression being targeted also contribute to immunological tolerance, a proportion of individuals treated develop inflammation which causes side effects and limits therapy. Discovery of such immunosuppressive mechanisms which do not contribute to immune regulation will enable more specific therapies to be developed, allowing, for example, disruption of cancer immunosuppression without induction of inflammatory disease in patients.

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

Therapies that work by targeting the immune system have brought about significant health benefits on a global scale. For example, therapies targeting immunosuppressive mechanisms in cancer are presently revolutionising the treatment for patients with metastatic disease.

Our research will extend our fundamental knowledge of how the function of the immune system is controlled not only to prevent otherwise deleterious autoimmune and allergic inflammation but also to limit effective immunity against chronic infections and cancer. The research will also provide a basis for the development of new therapies aimed at controlling immune function in patients with a variety of other disorders in which the immune system plays a critical role in inflammatory diseases and infections.

We will publish our research in peer-reviewed journals and present our findings ahead of publication as oral presentations and posters at national and international conferences.

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

Our research will be of benefit to researchers in related academic fields, such as cancer immunology, inflammatory disease biology and infectious diseases, and to researchers in the field of gene regulation and epigenetics. Aside from its relevance to academic researchers, the work is relevant to researchers aiming to make new therapies for individuals with immune-mediated disorders, cancer and chronic infection, including pre-clinical researchers and the pharmaceutical industry, with whom we have established collaborations.

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

The outputs of our research are primarily disseminated through research publications and presentations. Data and reagents are also directly shared with academic and industrial sector researchers ahead of publication. We also strive to engage the public with our science. We will seek opportunities to secure intellectual property and commercialise our research to foster UK indutrial 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.

Species and numbers of animals expected to be used

- Mice: 53780

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.

The behaviour of the immune system is poorly modelled *in vitro* (in a test tube, culture dish, or elsewhere outside a living organism), due to the complexity of the cellular interactions taking place *in vivo* (in a living organism). Hence we require an experimental system where homeostasis and disease can be modelled *in vivo*. The immune system in mice is similar enough to the immune system in humans that valuable parallels can be drawn. The availability of different genetically modified mice and reagents that recognise mouse cells means this species can be used more efficiently than any other species to ask questions about the role of particular genes in the immune system.

We usually use adult mice because the immune systems of adult mice are similar to humans and our research focuses on diseases which primarily affect adults. Apart from studying adult mice, we also need to study aged mice to investigate the immunoregulatory mechanisms which control inflammageing, an inflammatory condition developed in age.

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

A majority of mice will be used in the breeding of genetically-modified animals for experiments. A proportion of mice will be killed in a humane way so that organs can be analysed within the laboratory. Some 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 (intravenous, subcutaneous, intraperitoneal).

For example, we will test the immune response of mice bearing genetic alterations in the immune system to tumours by implanting small numbers of tumour cells under the skin of animals and letting them grow. Tumour growth will be monitored regularly by trained staff. Animals will not be allowed to suffer excessively, or beyond well-defined criteria. Animals likely to develop adverse effects that extend beyond the well-defined limits will be killed using a humane method. After 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 tumour immune responses are suppressed. In general, these experiments take around three weeks from tumour injection to euthanasia and animals may receive immunomodulatory substances during tumour growth to examine the therapeutic potential of targeting immunoregulatory mechanisms. Similarly, we will test the response of animals with genetic alterations in immunoregulatory mechanisms to acute infection, chronic infection, allergic asthma, and experimental autoimmune encephalomyelitis (EAE) using well-established disease models. The typical duration of such disease models is 2 weeks, 16 weeks, 4 weeks and 4 weeks respectively.

We also need to use models of cancer, and autoimmune and allergic inflammation to test immune function in animals which have received immune cells from another mouse. In such experiments, animals will typically receive an intravenous injection of immune cells and then infection, inflammation or cancer will be induced. Animals will be routinely assessed for signs of illness and weight loss and animals likely to breach the severity limits of the protocol will be euthanized.

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

A majority of animals will be used to breed and expand genetically altered mouse strains essential for the research, and will experience no significant adverse effects. Likely adverse effects from experimental procedures depend on the experimental model 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. These adverse effects are expected to last for 3 weeks, with significant ulceration triggering a decision to euthanize the animal. Animals under infection or inflammation models may lose weight and exhibit reduced activity. These adverse effects are expected to last for 2 weeks until the infection is cleared or the inflammation subsides. Animals induced with colitis are expected to have diarrhoea and blood is occasionally observed in the stool. These adverse effects are expected to last for 6 weeks.

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 92%

moderate 7%

severe 1%

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

- Killed

A retrospective assessment of these predicted harms will be due by 20 October 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?

Animals are necessary to understand how the immune system works because the various interactions of immune cells with other cells and substances in living animals cannot yet be generated in test tubes. Features such as the distribution of immune organs throughout the body and the ability of immune cells to migrate into almost all tissues of the body make investigations of the immune system in the

whole animal context essential. Adaptive immunity (the type of immunity which remembers previous exposures), which is the subject of this research, evolved in vertebrate animals and is not present in less sentient organisms. Among vertebrates, many cellular and molecular features are highly conserved between mice and men. Many useful tools and well-established experimental models for experiments in mice already exist, enabling 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 using cells cultured and expanded *in vitro* to test whether certain molecular pathways have an important role in controlling the immune system. These experiments while useful in replacing the need to use animals at the early stage of discovery, are very poor at modelling how the immune system works within the body.

Why were they not suitable?

In vitro experiments using purified immune cells fail to model the various interactions of immune cells with other cells and substances in the body. Moreover, such tissues contain complex mixtures of both immune and non-immune cell types each of which can signal to lymphocytes and affect their behaviour. 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 vitro*.

A retrospective assessment of replacement will be due by 20 October 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?

We have provided the estimated numbers of mice to be used under each protocol of the license. 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. 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.

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

We monitor 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 will 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 reduction in the numbers of animals bred.

Genome editing technologies such as CRISPR/Cas9 mediated gene editing offer a potential for reduction, since they allow the function of genes to be tested without germline mutagenesis and 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 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.

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. Where relevant, we will 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).

The use of IVIS live imaging will enable reduction of mouse usage by providing information on cell migration and immune response kinetics that cannot otherwise be obtained without euthanasia of animals and analysis of tissues at serial timepoints, providing a means for reduction of mouse usage.

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

We monitor the breeding performance of our mice to ensure that the minimum numbers of animals are used. We use colony management software that helps avoid overproduction. The use of inbred strains 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 matings. The number of 37000 reflects the number of mice required both at the crossing stages when different alleles are being combined onto the same background, and

at the colony expansion and maintenance stages. Our calculations were based on previous experience generating and maintaining multiallelic mouse strains.

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. Staff receive training in biostatistics. 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.

A retrospective assessment of reduction will be due by 20 October 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.

In general, suffering will be minimised by careful observation of the mice undergoing procedures and adherence to AWERB handbook guidelines for the assessment of clinical signs that will trigger the end of an experiment for a particular mouse or a cohort. Detailed written guidelines have been recently produced for the care of aged mice. Where an option of experimental models are available to answer a specific question, we will chose the model which causes the least suffering to animals.

Ageing mice: 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.

Choice of experimental models: For example, where tumour immune responses are being tested, we will primarily implant tumours by subcutaneous injection, also called the heterotopic tumour implantation model, and 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. It will also administer analgesia. In general, heterotopic models are preferable from an animal welfare perspective than orthotopic models (surgically implanting cells in recipient animals) and therefore heterotopic models form the mainstay of the proposed work involving tumour immunity although orthotopic models

in compliant and non-surgically implantable locations will also be used (eg melanoma tumours injected into the intradermal space, and breast carcinoma cells injected into the mammary fat pad).

Virus or bacterial infection: Infection with influenza virus or live bacteria will be carefully controlled to minimise adverse effects. Batches of infectious agents will be standardised using in vitro assays and the lowest dose sufficient to elicit the required immune response given. Additionally, we have established clear humane end points for infected animals to avoid unnecessary culling of infected animals in experimental groups.

Perioperative and post-operative analgesia: Perioperative and post-operative analgesia will be given when necessary using advice from the NACWO and NVS. Choice of analgesic, duration and dose will be adjusted to the clinical signs observed taking into account possible impacts on the experimental plan. Peri-operative analgesia will be given and maintained after surgery for as long as is necessary to alleviate pain. Analgesia will also be administered to control pain for painful procedures. In addition, procedures are refined to reduce the suffering that they cause and training of staff conducting procedures is maintained so as to minimise inter-operator variation and suffering.

Administration of tamoxifen: Administration of tamoxifen in the diet is known to cause neophobic effects in mice. We have gained experience 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.

Experimental autoimmune encephalomyelitis (EAE): EAE has a severe severity limit and is likely to cause substantial clinical signs in about 10-20% of the animals. It is necessary to use this experimental system because there are no other ways of modelling the relevant neuro-immune interactions underlying multiple sclerosis using less severe protocols or in vitro methods. To minimize animal suffering, we will carefully monitor the animals' condition, provide access to food and water in the case of immobility, and euthanize animals prior to exceeding severity thresholds. For example, we will provide DietGel and heat-pad to animals in the case of paralysis and/or weight loss. This model is widely recognised as one which most reliably allows measurement of autoimmune T cell-mediated tissue injury in the CNS. There is a great unmet clinical need in this area and our experiments could inform the development of new therapeutics.

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 programme of research 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 and 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.

Where it is necessary, we will monitor animals daily, such as by measuring weight and observing behaviour. We will keep close contact with animal technicians and kill animals once any significant clinical signs show.

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

Formation of ulcers is common in EAE models (Protocol 14). If mice develop ulcers < 3 mm in diameter measured by electronic callipers that do not resolve or continue to expand within 24 hours they will be treated with a topical hydrogel wound dressing as required, and if necessary antibiotics will be topically applied. Changes to ulcer size will be assessed daily by measurement and observation of the ulcer. Mice that have ulcers that continue to expand and/or increase in depth (> 24 hours) will immediately be given anti-inflammatory drugs in parallel with topical treatment.

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.

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 and 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 keep in touch with NVSs (Named Veterinary Surgeons) and NACWOs (Named Animal Care and Welfare Officers) and animal technicians for seeking advices when necessary.

A retrospective assessment of refinement will be due by 20 October 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?