Home Office

NON-TECHNICAL SUMMARY

# Immunity, Resilience and Repair

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

**Key words**

Immunity, infection, cancer, healthy ageing

| 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 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?**

We want to understand how lymphocytes interact with other cells types to promote immunity, resilience and repair at the organismal level. To achieve this we will combine physiological, cellular, molecular and computational approaches across the life course.

**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 immune system is now understood to be a collection of distinctive cell types that mediate immune effector functions, such as antibody production or cellular cytotoxicity, to promote immunity. However, the molecular basis for this, including the genes that promote the durability of immunity to infection and limit the effector functions of immune cells to avoid harmful reactions against self and promote resilience remains to be understood. The molecular and cellular basis of how immune cells promote the healing and repair process is essential to understand how these mechanisms operate optimally and deteriorate as organisms age and will be fundamental to treating the common human diseases of the 21st century.

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

We anticipate the principal outputs will be scientific publications. The intellectual property we develop may underpin the development of new therapeutic modalities.

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

Other Researchers:

Our work will create unique research tools, methods and data resources relevant to the development and function of lymphocytes. Through publications, presentations and the use of data repositories our work will influence other researchers, although this may not be fully realised until completion of the project.

Industry:

Our previous work on immunomodulation has a proven track record in translational outcomes and successful working with partners in small and large commercial enterprises. We anticipate continuing impacts and our findings will be commercialised, where possible, through collaboration with industrial partners.

Patients and Clinicians:

We will continue to ensure that our research models are relevant to prevention and treatment of human disease.

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

We will continue to publish our findings in peer-reviewed journals making use of open access, preprints and journals that accept negative findings or replication studies.

We will collaborate with colleagues in Universities, Medical Centres, other Institutes and the commercial/ biotechnology sector to promote awareness and influence the research direction of others.

We will deposit datasets and analytical tools in repositories and promote data accessibly through careful annotation, metadata and data visualisation applications.

We will share our research tools and know-how freely.

We will protect intellectual property and patent findings when appropriate.

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

- Mice: 108070

# 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 mouse immune system is extraordinarily similar to the human. While there are differences, these are far outweighed by the similarities; the fundamental appreciation that the cells and genes that regulate immunity in the mouse overlaps substantially with humans has brought fundamental health benefits in a global scale e.g., vaccination, skin grafts, therapeutic monoclonal antibodies, cell therapies. Remarkable discoveries such as regulatory T cells and subsets of innate lymphocytes were first made in mice and then found in humans. B and T cell receptors for antigen are formed by similar molecular processes and their repertoires selected and maintained by similar mechanisms.

The mouse has a long history of contributing to fundamental and applied immunology. The ability to delete individual genes in mice - in specific cells, at specific times - enables studies that are not possible in humans. The ability to perform infections or study the growth of defined tumours and take tissue samples for research is impossible or impractical with humans. Studies of controlled ageing cohorts under defined and constant environments are also impossible for researchers to perform over the human life-course.

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

Female mice (<2000 animals) will be injected with hormones to increase the production of embryos (superovulation) after which they will be killed to recover embryos (Protocol 1). To establish new mouse

strains a small number of female mice (<500) will receive embryos via surgical or non-surgical procedures (Protocol 2). A very small number of male mice (<20) will be vasectomised to produce pseudo-pregnant embryo recipients (Protocol 3), these males will be kept until twelve months of age.

We will breed and maintain genetically altered (GA) mouse strains for experimentation. The maintenance until a maximum of 15 months of age will cover the mice used in this study under Protocol 4 where no or rare (<10%) mild effects are anticipated (< 84000 procedures). The majority of mice of both sexes will be studied either after killing by a humane method to address questions on lymphocyte homeostasis, or under the experimental protocols below to study lymphocyte responses. We will recover post-mortem tissues and purify and/or grow lymphocytes for use in the studies below.

Some GA mouse strains may manifest moderate clinical signs as a consequence of the alterations to their genomes. Protocol 5 (< 150 animals) enables us to address questions of immune homeostasis in mice that may develop clinical signs that exceed mild limits of protocol 4 such as marked piloerection, intermittent hunched appearance, abdominal distension. We have a good understanding of the age of onset of clinical symptoms and when clinical features arise we shall aim to euthanise mice to recover tissues within 24 hours of symptoms being reported, or before exceeding moderate severity, to avoid suffering. Some mice with conditional Myc expression, a PI3K E1020K mutation or BCL6 transgenes may develop lymphoma. We have designed our breeding programme to minimise the numbers of mice that have an oncogenic combination of transgenes. We freeze bone marrow from these mice to use as donor cells for transplantation experiments under protocol 10 to generate a cohort of experimental animals in which the onset of disease is more synchronous.

A minority of mice including inbred strains with no genetic modification with no or mild phenotypes will be aged beyond 15 months (no more than 1300) to enable us to study changes in immunological processes with age (Protocol 6).

One important experimental approach we use, common to several of the following protocols is to use inducible gene deletion. Tissue specific and temporal gene regulation in cells can be achieved using a number of genetic alterations. These studies will involve a minority (~15%) of all procedures.

For the majority of our studies of immunity we will challenge the immune system using non-replicating antigens which trigger lymphocytes through their antigen receptors together with adjuvants or other immunomodulators (Protocols 7: 16,900 animals 7 and 8: 2000 animals).

The distinction between these protocols is that Protocol 7 will use genetically altered mice that have been bred specifically for the protocol, or mice into which we have transferred cells (adoptive transfer) to study how well they perform their specialised functions and how they influence other cells in the host; while Protocol 8 will use mice that have been prepared by stem cell transfer into irradiated recipient mice. The fewer than 2000 mice studied under Protocol 8 need 8-12 weeks to reconstitute the immune system before they can be used for studies of homeostasis or immunity.

When we use infectious agents that replicate, we will prioritise the use of attenuated bacterial or viral stains, live vaccines, or expose to doses of infectious agent from which we expect the animals to recover after experiencing moderate severity. This may result in some discomfort similar in duration and severity similar to that of a vaccination or infection, but is rarely found to lead to severe outcomes.

Under Protocol 9 mice will be inoculated with tumours which will grow for up to two weeks before administration of immune cells and subsequent study of the effect this has on tumour growth. These experiments should not last longer than 32 days and the numbers of animals used will be 500.

The study of the development of lymphoma will be conducted under Protocol 10 (< 400 animals). Frozen bone marrow or other sources of stem cells will be used to reconstitute the haematopoietic (blood cell) systems of irradiated mice. These will be monitored until the onset of clinical signs which is usually within six months of reconstitution.

In order to be able to investigate the ability of T cell subsets to regulate a graft versus host (GvH) response, Protocol 11 will use a proven allograft model using T cell-depleted bone marrow from C57BL/6 mice transferred into lethally irradiated Balb/c recipients and co-transfer of T cell subsets to elicit an immune response (<300 animals). These experiments will typically last less than 60 days.

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

For most of the mice, including immunodeficient strains, we do not expect any impacts or adverse effects in our high-quality AAALAC accredited specific pathogen-free (SPF) animal care facility. NOD SCID Gamma (NSG) immunodeficient mice mice can exhibit progressive hearing loss that can be profound at three months of age.

Embryo transfer and vasectomy are surgical procedures with short term post-surgical pain. Post-surgical pain will be controlled by giving pain relief and any animal not fully recovered (eating, drinking, return to normal behaviour) within 24 hours will be euthanised.

For some GA animals bred on this licence there will be adverse events associated with loss of immune homeostasis. These can manifest as lack of weight gain, or even weight loss. Other visible impacts of inflammation include intermittent hunching, piloerection, deterioration of coat condition, a reduction in activity, abdominal distension, scaly/scurfy skin on ear, around eyes and on tails.

Although ageing is a major risk factor for adverse effects, we know that the vast majority of our aged mice remain healthy throughout the duration of their lifetime. There is an increased incidence of adverse effects not observed in young wild-type mice including altered coat condition, diarrhoea, eye abnormalities, abdominal distension, movement issues, tremors and seizures. A tiny minority of these develop tumours, but regular checking by our experienced animal technicians ensures these are detected early, and the mouse euthanised immediately. A specific code of practice for caring for aged mice is in place.

The adverse effects of irradiation can be infection or failure to repopulate the haematopoietic system. These effects, when they arise, are manifested within the first two weeks following irradiation, most conspicuously as anaemia and weight loss. Some mice may lose pigmentation as a longer-term impact of irradiation.

The adverse effects of immunisation/infection or immunomodulation include systemic or specific tissue inflammation which will be transient, lasting for a few days. In the case of influenza virus there will be substantial weight loss which is restored within two weeks. Intermittent abnormal breathing may occur, but this will be transient lasting only a few days.

Some viruses will induce chronic infections with adverse effects such as weight loss and while this may be moderate in C57BL/6 other inbred strains manifest enhanced vascular permeability, lung immunopathology and animals will be closely monitored according to the humane endpoints detailed.

Tumour inoculation may lead to organ failure or affect breathing, feeding or drinking. Transplantation of human cells into non-irradiated NSG immunodeficient mice can lead to graft versus host disease (GvHD), but adverse effects will be reduced by introducing a genetically engineered Chimeric antigen receptor (CAR) into the transferred T cells. Cytokine release syndromes and neurotoxicity associated with these tumour immune models manifest as reduced activity, piloerection and weight loss.

The harmful manifestations of lymphoma may include reduced activity, intermittent hunching, marked piloerection, intermittent diarrhoea and occasionally intermittent abnormal breathing.

The adverse effects of GvHD include weight loss, which can become severe if not closely monitored. Signs of inflammation such as intermittent hunching, piloerection, flaking or reddening of the skin are also likely within days of the onset of GvHD.

**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)?**

The expected severity for most of the mice will be mild or sub threshold

- Total animals used =  108070
- Mild: 24% (26440)
- Moderate: 2% (1830)
- Sub threshold: 74% (79800)

**What will happen to animals at the end of 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?**

Animal experimentation is necessary because we want to understand the systemic properties of immune cells at the organismal level.

While we recognise limitations of mouse models and the need, where possible, to verify findings in other systems and species, we emphasise the mouse has proven overwhelmingly successful for the discovery of new cell types and the molecular genetic mechanisms underpinning their function. It has yielded essential basic knowledge of the immune system now being applied to improve the health of the humans and economically-important mammals.

Furthermore, it is extremely difficult to study lymphocytes in the bone marrow or lymphoid tissues of humans, because it is very difficult to obtain these from healthy donors.

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

We have considered using cell culture methods, including organoids and do use these when appropriate. For example, we are using an *in vitro* model to investigate molecular pathways involved in germinal centre formation and plasma cell differentiation to complement the *in vivo* studies.

**Why were they not suitable?**

Features such as the distribution of lymphoid organs throughout the body and the intrinsic properties of lymphocyte recirculation cannot be recapitulated in tissue culture or organoids making investigations in the whole animal context essential.

# 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 estimated the number of animals we will use based on our previous studies using these protocols. The numbers of mice required for the generation and rederivations of genetically altered mice are based on extensive experience of staff who regularly perform these protocols.

The use of colony management software and knowledge of the breeding performance of individual strains has enabled us to predict the numbers of mice of the correct genotype that we will produce from breeding, and the numbers of aged mice that we will need.

The numbers of mice required for experimental groups are based on power calculations, appreciation of variability and a knowledge of biologically meaningful effect sizes. We have factored in the need for experiments to be replicated independently and for greater variation in the responses of aged mice.

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

We reduce the numbers of animals used by paying careful attention to the design and planned analysis of the results including consultation when needed with a biological statistician in the Bioinformatics department and we use the NC3R's Experimental Design Assistant to ensure we are considering all relevant aspects of design. We perform our experiments in carefully controlled animal facilities that reduce biological and environmental variation; the use of optimised experimental procedures, including the use of genetically identical strains, blinding and randomisation to reduce technical variation; the multiparameter analysis of individual mice; the adaptation of new technologies such as the use of ribonucleoproteins to create knockouts or other genetic modifications; improving the sensitivity of techniques to enable measurement to be made on small cell numbers isolated from a single animal. For some experiments we are using in vitro methods to promote cell differentiation to defined states under controlled conditions, thus enabling access to cell numbers that would be unfeasible to obtain from mouse tissues.

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

Where possible we will share mouse strains rather than generate new strains by transgenesis. Our mouse facility has an extremely efficient pipeline of tissue biopsies and rapid genotyping by a commercial provider, that provides timely results to enable efficient breeding.

Selective deletion of individual genes in specific tissues will be achieved by the use of Cre-Lox recombination to delete the gene of interest in defined cells. We employ rigorous quality control to ensure the fidelity of these systems. Pilot studies ensure minimum use of mice before decision points are reached in experimental design.

We have an established and highly successful programme of sharing of tissues with other investigators which we will continue.

We will prepare frozen stores of biological samples (tissue cells from infection and immunisation studies; bone-marrow; serum; and tissue sections) so that experiments can be performed on previously gathered tissues rather than using new mice.

# 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.**

This project will use young-, adult- and aged-mice up to 26 months that are wild-type, transgenic, gene-targeted or contain reporter genes.

The small number of animals that will be embryo recipients, or undergo vasectomy, will experience transient pain due to surgery. This will be ameliorated by analgesia.

GA mice usually have a single gene altered, but multiple genes may be altered in the case of genetic redundancy or to combine gene mutation with reporters of cell fate. Typically, mutation is achieved by conditional deletion thus sparing any harmful or unwanted side effects of mutating a gene in all cell types of the body. In many cases, there is no pain, suffering, distress, or lasting harm expected with these models. In our facility which is pathogen free the animals with modified immune cells can live for normal lifespans without harmful effects of infection. The exception to this is for a small number of animals with abnormal immune regulation that may experience some symptoms of autoimmune or inflammatory disease - where mice displaying clinical signs will be killed and used for tissue collection before clinical signs exceed moderate severity.

Some animals will experience infections or immune challenges that will elicit immune responses. These experiments will be further refined by using Cas9-based methods to inactivate genes in cultured primary lymphocytes. This will refine some experiments in which cre-expression may cause unwanted effects that are difficult to control for. It will also reduce the numbers of animals bred.

The use of non-replicating antigens, vaccines, and attenuated microorganisms and in some cases vaccine strains (which have limited pathogenicity) will enable questions about immune responses to be answered.

Some experiments will use protein degron technology (where short amino acid sequences - as degradation signals - are used to manipulate protein degradation) as an alternative to genetic deletion of the gene. This important innovation allows protein ablation, but unlike gene ablation it is revertible. Moreover, it is not limited by the features of the cre-lox system which requires Cre to be expressed at a specific time.

Some experiments will involve injecting sources of haematopoietic cells to reconstitute the immune system. These experiments provide detailed *in vivo* knowledge that studying cells *ex vivo* cannot do. This can occasionally lead to ill health, either as a consequence of the irradiation or graft versus host disease, but these mice will be carefully monitored and any animal exhibiting moderate pain or distress will be culled.

We will use a specific type of immunodeficient mice (NSG) for transplantation of human tumour cells as these are considered to be a relevant preclinical model for adoptive cell therapy.

Suffering will be minimised by provision of analgesia where appropriate, provision of diet-gel food on cage floors should animals have difficulty accessing water due to mobility impairment, sub-cutaneous hydration if dehydration is apparent, housing in heat room/on heat pad if temperature drops significantly (e.g., following anaesthesia).

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

Importantly, 'less sentient' species are not applicable for studies of B and T cells and the choice of species is limited by the fact that in evolutionary terms the adaptive immune system is largely a vertebrate invention. Even so, in some vertebrate species such as Zebrafish, the properties of

lymphocytes differ significantly from humans and mice making them interesting but unsuitable for our project.

The mouse is the species of choice because of the extensively validated tools and resources available for quantitative immunological phenotyping and mechanistic studies across the life-course. Studies of ageing in the mouse have provided key insights into human immune systems but require the long-term care of mice with which we have much experience. The availability of inbred stains and well annotated genomes is important for our studies as they develop into the investigation of molecular mechanisms *ex vivo* and *in vitro*. The requires careful and attentive monitoring of breeding programmes and quality control.

Early-stage mouse embryos are unsuited to these studies as the adaptive immune system and immunological memory is a feature of adult animals and we propose to study these features in adulthood and ageing animals.

Terminal anaesthesia is inappropriate for long-term experiments that require systemic immune responses, but may be used to prepare tissues for further study.

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

We are mindful of the increased incidence of poor health for some individual mice as they age and that this may be affected by genetic modification, which may have a positive or negative impact on this process.  We observe these mice with increased frequency and have developed a detailed checklist of possible age-related changes, and procedures for monitoring and treatment. Data gathered from these studies will be available to further refine best practice.

We will implement any refinements developed by our animal house staff, who have a long history of innovative practice, including environmental enrichment.

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

All experiments which will integrate refinements from the NC3Rs such as the ARRIVE guidelines on design and reporting; the LASA aseptic guidelines; LASA Diehl guidelines on volumes and frequency limits and the most up-to-date veterinary knowledge.

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

We will stay informed about advances in the 3Rs during this project through attention to the work and outputs of the NC3Rs including workshops and webinars; by invited seminars on the 3Rs; and seminars on Research Integrity - which covers experimental design and data management. We will actively stay updated with our field of research through collaboration, conference attendance and reading the literature which frequently highlights innovations (e.g., Cas9 methods). We will use Home office advice made available to us through our dedicated Home Office Liaison.

To implement advances effectively, we will follow guidance from our local AWERB. Our animal facility also has a dedicated Strategy Committee and a User group, into which we influence and are made aware of emerging best practice. These groups discuss and make collective decisions about advances in the 3Rs and advise on how they can be implemented, both across the organisation, and by individual researchers.