Home Office

NON-TECHNICAL SUMMARY

Neutrophils in infection and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

infection, neutrophil, macrophage, inflammation, cancer

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

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?

This project will study the mechanisms that regulate inflammation focusing on neutrophil and macrophage responses and how they are affected by changes in hematopoiesis during infection, chronic inflammatory disease and cancer.

A retrospective assessment of these aims will be due by 30 May 2026

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?

Neutrophils are crucial for immune defence but are also driving immune pathology when they become deregulated. Severe infections, cancer and atherosclerosis are the major drivers of premature death worldwide. Neutrophils play a critical role in all of these diseases. Understanding how neutrophils protect against infection and more importantly how they are implicated in sepsis, cancer and atherosclerosis and other inflammatory conditions will provide new avenues of treatment for diseases that remain largely untreatable.

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

This project aims at uncovering novel mechanisms that drive sepsis, cancer and chronic inflammatory disease that could be targeted therapeutically in order to improve the lives of patients suffering from these diseases. In addition, it aims at addressing fundamental questions about the functions of neutrophils in immunity and disease. This work will be published in a number of scientific research publications and collectively present an inter-connected body of work that will advance our understanding of the mechanisms that fine-tune the production and function of neutrophils and regulate their interactions with the other parts of the immune system.

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

In the short-term (2-5 years): academics, researchers in academic institutions and the pharmaceutical industry. In the long run (5+ years) inflammatory disease, infection and cancer patients, clinicians and the general public.

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

Presentation in national and international scientific meetings and seminars at other research institutions.

Publication of high impact research papers, reviews and book chapters.

Collaboration with other basic and clinical researchers.

Disseminations via public engagement activities.

Species and numbers of animals expected to be used

- Mice: 25000

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.

We work with mice as they are well-characterized model organisms to study the immune system and model human infection and disease. In addition, countless critical genetic resources have been developed in mice that are invaluable for our work.

Nearly all of our immunological challenges are tested on young adult mice which have robust and mature immune systems.

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

The protocols in our project employ a number of procedures such as injections or administrations of microbes, immune or tumour cells of small molecule substances, intravenously, orally or intratracheally. Moreover, some mice receive an altered diet, such as a western style high fat diet. In one protocol mice will undergo a minor surgical procedure to inject tumour cells into the pancreas. Depending on the experimental design experiments with microbial challenge last approximately 1 week. some mice may be infected on multiple occasions with small doses of microbes to evaluate the effect of chronic low grade exposure on tissues and immune cells. Some mice may be infected with higher doses that will cause pneumonia or a skin abscess. Other mice may receive microbes systemically that cause septic shock in order to understand the mechanisms that promote the condition as well as the mechanisms that protect against its onset. Tumour challenges last approximately 2 weeks, arthritis models 10 days and atherosclerosis dieting typically 6-12 weeks. Animals may also be induced to develop arthritis that spontaneously resolves after 2 weeks.

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

Infections typically cause weight loss and some transient discomfort characterised by reduced activity. Skin infections may cause abscesses that in some cases may ulcerate resulting in the animals having to be sacrificed. In more severe cases such as sepsis models, mice may experience systemic inflammation with some pain, lethargy, drop in body temperature and respiratory distress, which will be endpoints in the experiment. However, monitoring the temperature allows us to cull the animals before they reach these severe symptoms if meaningful results can be obtained in earlier stages of the condition. Tumours do not usually cause visible symptoms but some weight loss may occur. Large metastatic tumours may cause more significant weight loss and respiratory problems which will be endpoints in our experiments The atherosclerosis model does not cause any visible harm aside from weight gain and vascular plaques that are asymptomatic. The arthritis model will cause some limb swelling and may impact on mouse mobility to some degree.

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 majority of our protocols are of mild or moderate severity. Only one protocol employing a model of microbial sepsis is classified as a severe protocol as mice are expected to develop symptoms associated with systemic hyperinflammation. However, the mice will be closely monitored and will be sacrificed as soon as these symptoms begin to appear.

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

- Killed

A retrospective assessment of these predicted harms will be due by 30 May 2026

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?

Our projects investigate the functional and mechanistic basis for inflammatory diseases. While certain aspects can be addressed using in vitro cultured experiments, to establish the relevance in vivo and to dissect the events that drive these diseases functionally in in vivo requires the use of animal models of disease, particularly since none of the diseases we are studying can be fully recapitulated and modelled in vitro using organoids. Sepsis is a complex disease implicating several different organs and

cell types. Similarly, atherosclerosis and the interactions of immune cells with tumours must also be examined in their native in vivo environment.

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

We are employing human primary neutrophils and other myeloid cells to conduct many of our mechanistic experiments before proceeding to in vivo validation. We are also conducting descriptive studies with human clinical samples of sepsis and atherosclerosis. This approach reduces the number of mice we use in our projects. However, for certain projects as in sepsis we have relied on mouse experiments guiding subsequent mechanistic in vitro studies. The only real alternative to in vivo studies are organoids but they are not applicable to the diseases we are studying.

Why were they not suitable?

All of the aforementioned approaches are complementary to in vivo experiments, but unfortunately cannot replace animal work given that the questions that we are investigating have to be examined in the native disease context.

A retrospective assessment of replacement will be due by 30 May 2026

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?

Pilot experiments and biological replicates

When setting up a new experiment, rather than using large groups of animals and having to repeat these large-scale experiments to refine the experimental parameters, we start with very small groups (2-3 animals /group) and test several parameters we predict to be important for optimization. Subsequently, we follow up the selected conditions that provide good experimental data with additional experiments consisting of small groups that we add to the original study until we reach statistical significance. This phased sequential experimental design reduces the overall number of experimental animals used in our studies since it limits the number of animals that participate in studies under sub-optimal conditions.

In addition, we keep experimental groups small and divide mice over a larger number of independent experiments (biological replicates) in order to obtain better statistical significance from a smaller total number of experimental mice.

Power analysis

Prior to designing experiments we use published data and our own past experience to set the appropriate sample sizes. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 20%.

Imaging

We are also implementing imaging techniques that in many cases are non-invasive and allow us to monitor the progression of infection, inflammation and tumour growth, without sacrificing animals. In this manner we can constantly monitor experimental animals and select optimal timepoints to terminate experiments rather than using multiple animal cohorts sacrificed at different timepoints in order to capture the best timepoint for measuring parameters. This approach minimizes the premature termination of experiments which would require unnecessary repetition and prevents unnecessary suffering by not allowing experiments to proceed beyond the optimum time point.

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

As specified above, we conduct small scale pilot experiments to guide power calculations prior to expanding our studies. In many cases long term experiments are performed with in smaller groups and in a phased manner, allowing us to increase the total sample size progressively until the required statistical power is achieved. The early results of the magnitude of changes between experimental groups are filtered through online power calculation tools to estimate accurately the sample sizes required for publication. Through small sample sizes and pilot experiments we can also refine variables like microbial and treatment doses for subsequent experiments

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

1. Breeding: We monitor the breeding of our mice closely to ensure that only breed the number of animals we need for experiments without breeding excess animals that will not be used in any experimental protocols.

2. Multiple uses of tissues, future proofing: From each experiment we collect the maximum number of tissues, even though we may not need them all for the purposes of the present experiment. Given that we employ standard experiments in many of our projects, we keep frozen tissues and samples from all these mice and organise them in a database. This allows us to easily access the tissues in the future without needing to replicate the experiment with new mice. This practice has reduced the number of experiments we have been conducting in the past.

3. Small pilot studies always inform our group sizes, as well as avoid unnecessary large scale experiments that do not show promising results in the small-scale pilot studies. If pilot studies fail to demonstrate signs that experiments may yield interesting data, then no follow up large scale experiments will be conducted and projects will take other more promising directions.

A retrospective assessment of reduction will be due by 30 May 2026

The PPL holder will be required to disclose:

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

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will employ several murine models of pulmonary and systemic infection using fungal and bacterial pathogens. We will also employ two models of sterile chronic inflammatory disease: murine atherosclerosis using the administration of high-fat diet and a transient model of rheumatoid arthritis using injection of auto-reactive antibodies against collagen. Finally, we will employ several models of primary and metastatic tumours.

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

These murine models are optimised to cause the least amount of suffering. They last anywhere from 24 hrs to several weeks and therefore the animals cannot be anaesthetised for this period of time. However, they are temporarily anaesthetised when undergoing certain procedures. Murine models of disease are very similar to human disease and recapitulate many of the attributes and mechanisms of pathology observed in human patients. They also have a very similar immune system with that of humans that is extensively characterised. In addition, over several decade a multitude of genetic tools and reagents that are specific for mice have been developed that are essential for our research.

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

Over the years we have refined several of our protocols, particularly by fine-tunning the rate of monitoring and infection doses in our severe sepsis protocol which allow us to predict relatively accurately when WT and GA mice will develop sepsis. We have also refined the breeding pairs needed for maximal use of mice in experimental protocols. These are constantly adjusted according to

experimental needs. The time-courses for atherosclerosis experiments have also been well characterised from our prior work, allowing us to estimate with accuracy the length of time allowed to obtain the degree of plaque formation needed for specific experiments.

We have also refine our anaesthetisation protocols which reduced the recovery time for mice and reduced the appearance of unexpected symptoms in response to infection.

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

We are aware of NC3Rs. We also discuss with colleagues in other research groups new improvements that lead to refinement. In addition we follow the literature and improvements in commercial reagents in a constant search for more efficient and better refined alternatives. This has led us to improve our arthritis model which is now 100% penetrant, requires fewer injections and much less time and is much more predictable and consistent than the traditional immunisation protocol.

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

Our institute regularly distributes newsletters and holds seminars to inform us on 3Rs and ways of improving our methods reducing the number of mice we use and refining our techniques both en mass and at a personal level. We also regularly seek advice from our veterinarian on how to improve our procedures.

A retrospective assessment of refinement will be due by 30 May 2026

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?