Home Office

NON-TECHNICAL SUMMARY

Immunopathology of experimental blood-stage malaria

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

malaria, immunopathology, brain, treatment, inflammation

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to improve our understanding of the pathways and processes that control the activation of the immune system and cause severe disease during malaria.

A retrospective assessment of these aims will be due by 28 February 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to undertake this work as malaria still causes significant illness and death in developing countries throughout the world. Animal models provide critical opportunities to identify and mechanistically test the processes and pathways responsible for promoting severe malarial disease, using procedures that are impossible to perform in humans.

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

The work in this project will lead to significant new information on the pathways and processes that activate and regulate the immune system and which promote pathology during malaria. Direct outputs from the work will be peer-reviewed research articles, dataset resources that will be shared with the research community, and presentations, where we will disseminate our discoveries.

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

The outlined programme of work will provide new insight into the most appropriate molecules and pathways to target as treatments for severe malarial disease, in particular cerebral malaria, which is the most severe complication of malaria that causes brain pathology. This will be of major benefit to researchers working on human malaria and should, in the short and mid-term future, direct clinical trials of therapies for cerebral malaria, which will ultimately be of benefit to millions of individuals in malaria-endemic regions of the world.

In addition, by dissecting the activation and regulation of the immune system during malaria, our work will demonstrate how to therapeutically manipulate the immune response against *Plasmodium* spp. parasites (the causative agent of malaria), which in the mid-term will have impact for strategies to augment protective memory responses to malaria and improve vaccine designs for malaria.

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

We will publish our results in peer-reviewed journals, in open-access format when possible. We will also initiate new and build upon existing collaborations to enhance the impact of our results. We will disseminate unsuccessful approaches or negative data through specific journals or online forums.

Species and numbers of animals expected to be used

- Mice: 2850

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.

Mice are the most appropriate species for this work as murine malaria infections are the most well-characterised of the various animal models (when using established parasite lines, as will be done in most experiments within this licence), and there is a significant body of literature, including from ourselves, that results obtained in murine malaria studies are relevant for understanding human malaria. We will utilise young adult mice as we require that the immune system is fully formed so we can appropriately translate results from mice to humans.

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

The general project plan will involve infecting mice with different species of *Plasmodium* parasites that cause specific types of malarial disease - ranging from mild malaria to cerebral malaria (a severe syndrome of malaria that affects the brain). The course of infection will be monitored by following peripheral parasite levels, through obtaining drops of blood from the tail vein. Depending on the question addressed in each experiment, mice may receive injections to modulate the immune system or physiological processes, may undergo surgery to modify tissue function (e.g. removal of the spleen to influence the immune system, or ligation of brain lymphatic vessels to change how cells and molecules drain out of the brain), or may receive anti-malarial drugs to kill parasites. Injections can be by different routes depending on the research question and the nature of reagents administered (i.e. reagents may be injected directly into the brain or provided systemically into the blood). The vast majority of animals will recieve less than 4 injections to manipulate the immune system or physiological processes, by a maximum of two different routes. The experiments will be typically short duration of 7 -14 days when assessing the immune response and level of pathology during primary *Plasmodium* infections; however some experiments may be > 60 days, when studying the development and activity of memory

immune cells (the cells that are maintained post-infection or vaccination to provide protection against subsequent infection). In some experiments, animals may be re-infected with *Plasmodium* parasites after clearing a previous infection to assess how repeated infection influences parasite control and the development of severe malarial syndromes. Multiple manipulations in a single animal will be avoided, when possible. Cumulative effects (e.g. additive effects) of multiple treatments will be minimised by allowing animals to fully recover from any serious procedure (i.e. surgery, irradiation and reconstitution) before the animals undergo any subsequent treatments.

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

Depending upon the species and strain of *Plasmodium* parasite and the strain of mice utilised, malaria infection may lead to mild, moderate or potentially severe suffering. Mild suffering occurs due to activation of the immune system and the general feeling of malaise (e.g. lethargy, fever or aches) that is associated with infection. Severe suffering during malaria occurs due to weight loss and loss of circulating red blood cells (anaemia) and / or damage to the tissues in the body (such as the brain during the development of cerebral malaria). In particular, during cerebral malaria the damage to the brain causes the tissue to swell, which causes pain to the animal and may lead to fitting and / or coma. However, of the experiments involving infections that have the potential to cause severe suffering in animals, not all infections will be allowed to progress to the stage where severe suffering occurs (i.e. experiments will be terminated at early stages before severe malaria develops to allow us to define the factors responsible for development of disease, or animals will be treated with anti-malarial drugs to terminate the infection). Most of the procedures performed or the reagents administered should not directly promote animal suffering. Animal suffering will be minimised by closely monitoring all animals in relation to a well-defined grading system and providing analgesia, when required and when possible without negatively impacting the course of the experiment. All administrations will be performed via the most appropriate route through (when applicable) the careful control of injections. Using our well-defined grading systems, of the animals that may experience severe suffering during the course of our experiments, suffering will typically be less than 4 h and not more than 12 h.

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)?

Experimental animals will be routinely monitored during the course of infection and the severity of disease and level of suffering will be graded according to well-defined scoring systems.

In infections that do not cause cerebral malaria, we expect 25% of animals may experience short-term (<24 h) moderate suffering (principally evidenced by lethargy or hyperventilation).

For infections that cause experimental cerebral malaria, the majority (>50%) of mice on this protocol will experience short-term (<12 h) severe levels of suffering (principally evidenced by hunching, respiratory distress and reduced responsiveness to stimulation).

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

- Killed

- Used in other projects

A retrospective assessment of these predicted harms will be due by 28 February 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We can only address the majority of our questions when a complete immune system is present in its normal anatomical and physiological configuration (for example within the spleen, the major site of immune priming and parasite killing during malaria infection), or when parasites and immune cells can interact with the complex architecture of the intact brain (leading to cerebral malaria): the use of animals is, to a significant extent, unavoidable in our experiments.

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

When we have simple and reductionist questions, such as how parasites directly interact with brain endothelial cells, then we can establish *in vitro* co-culture systems to study this interaction.

Why were they not suitable?

Such *in vitro* co-culture approaches are suitable for only very specific questions as during the course of a normal infection *in vivo*, the interaction between parasites and brain endothelial cells is shaped by a myriad of factors, including circulating immune cells and immunological mediators, and the multi-faceted communication with other brain resident cells. Thus, for the majority of our investigations to obtain accurate and physiologically relevant results, we need to study our objectives within intact tissues, *in vivo* or *ex vivo*.

A retrospective assessment of replacement will be due by 28 February 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

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

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

We have estimated the numbers of animals based upon our previous experience of running similar project licences in the last 15 years. Thus we have accounted for the nature and requirement of the projects we are currently working on, including the numbers of times experiments must be repeated, the numbers of different experimental groups in experiments, and the numbers of mice required in different groups. We have also estimated the number of animals to be used based upon future plans and collaborations.

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

We calculate the required group size using data from previous experience, and published work. This ensures that we have sufficient power to detect a biologically relevant effect using as few animals as possible. We also perform sample size calculations based upon pilot and preliminary experiments to ensure we perform subsequent experiments with the correct number of mice to detect statistically significant results. We also adhere to ARRIVE and PREPARE guidelines for reporting of research involving animals, which outlines appropriate study design (e.g. control groups and sample sizes), how to avoid experimental bias, and the analytical framework for simple and complex experiments..

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 perform pilot experiments when undertaking new experimental approaches so we can discontinue uninformative or inappropriate methodologies and so we can also evaluate the variability and magnitude of experimental effects. This will allow us to accurately assess the numbers of mice to use in future studies. We also consult the literature when we are performing similar approaches as others have previously performed, in malaria or in other models. This allows us to predict the strength of expected effects within our experiments, and therefore, the numbers of mice that need to be used to detect statistically and biologically relevant results. We will carefully manage maintained colonies (i.e. by employing short-term harem breeding) to ensure we have sufficient numbers of mice for planned experiments but ensuring we do not have surplus mice. Any unneeded mice will be shared with researchers, who have authority to receive animals.

A retrospective assessment of reduction will be due by 28 February 2027

The PPL holder will be required to disclose:

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

Refinement

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

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

Mice are the most appropriate species for this work as murine malaria infections are the most well-characterised of the various animal models (when using established parasite clones, as will be done in most experiments within this licence), giving us essential background information that is lacking in other systems. For example, in a previous project licence we performed important comparative assessments of the murine cerebral malaria model with human cerebral malaria, to evaluate the relative merits and translational utility of the murine model for studying the pathogenesis of human malaria, and for identifying the events that determine the anti-malarial drug treatment effectiveness of the syndrome.

The only alternative experimental models of mammalian malaria infections are non-human primate models, involving monkeys or apes. Mice are also the animals of choice for immunological investigations as so much is known about their immune systems, different well-characterised inbred strains of mice exist with differing responses to infection, there are a large number of genetically modified murine strains available for use, and all the reagents that we require (such as for modulation of the immune system) are available. Lastly, mice are well-adapted to captive environments.

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

To obtain informative results in this project we need to utilise a warm-blooded mammalian host that can be infected with evolutionary adapted *Plasmodium* spp. parasites and where the biology of Plasmodium infection is comparable to that in humans. This precludes the use of less sentient Zebrafish or drosophila models. We must also use adult mice with a fully formed and functional immune system. Otherwise, our results would be difficult to translate to the study of human malaria. We will perform certain protocols under terminal anaesthesia but due to the length and course of experimental malarial infections, it is not possible to perform all work under anaesthesia.

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

Due to the overall purpose of this work - to study the factors responsible for development of severe malarial disease - we do need to allow experiments to proceed to the point where animals will experience some suffering, recapitulating the development of severe malaria in humans. However, through using our well-defined grading system, of the animals that may experience severe suffering during the course of our experiments, we will ensure that none of these animals will experience prolonged suffering for more than a few hours (generally less than 4 h and no more than 12 h). Moreover, animal suffering will be minimised by providing analgesia, when possible and when

required. For example, whilst we can provide analgesia following surgery, we are unable to provide analgesia during the course of infection or to mitigate the effects of cerebral malaria, as the analgesia itself will modify animal behaviour and the course of the experiment. Multiple treatments to manipulate the immune system or physiological process within a single animal will be avoided, when possible, with a maximum of two separate approaches applied in any animal.

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

We will follow NC3Rs and LASA guidance and we will continually assess our experimental designs in relation to advances within the relevant malaria and immunology literature.

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

We are on the NC3Rs mailing list where we obtain newsletters with new advice and guidelines and information from other sources (such as LASA) are also communicated to us via email announcements. Standard operating procedures for users working with animals are in place within our institution, the adherence to which is compulsory, which incorporates advances in animal handling and ensures animal welfare.

A retrospective assessment of refinement will be due by 28 February 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?