Home Office

NON-TECHNICAL SUMMARY

Further Development of Anti-malarial Transmission Blocking Interventions

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, Plasmodium, Transmission, Mosquito, Vaccine

Animal types	Life stages
Mice	Adult

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?

This project aims to discover how malaria transmission occurs via infection with the Plasmodium parasite, and develop novel ways to prevent transmission to mosquitoes, reducing disease.

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 project aims to support global efforts to control and ultimately eliminate malaria. Malaria remains a significant public health challenge: an estimated nearly half of the world's population (approximately 2.2–2.4 billion people) remains at risk of infection, with approximately 282 million new malaria cases and about 610,000 deaths recorded worldwide in 2024. The vast majority of these deaths occur in sub-Saharan Africa, where roughly 94% of cases and 95% of deaths are concentrated, and young children under five years of age account for a disproportionate share of mortality. These evolving disease dynamics, together with concerns about drug and insecticide resistance, climate change, and gaps in funding and health services, underscore continuing gaps in global malaria control and elimination efforts.

The development of novel strategies that block malaria transmission is a high priority for scientists, funders and policy makers, who recognise the need to complement and extend the impact of currently utilised malaria interventions. By focusing specifically on the mechanisms by which the *Plasmodium* parasite transitions between mosquito and human hosts, this project seeks to identify new vaccine candidates or drug targets within the sexual stages of the parasite lifecycle. Interrupting transmission at this critical stage would prevent completion of the *Plasmodium* lifecycle and thereby reduce the incidence of disease. In addition to applied translational goals, the project includes fundamental research into parasite biology to deepen our understanding of malaria disease and support long-term global elimination objectives.

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

Drawing on our established collaborations with pharmaceutical partners, in-depth expertise in parasite biology, and extensive experience across multiple related projects, we are confident in our ability to successfully deliver the overarching objectives of this proposal. Specifically, the project aims to:

Identify approximately 15 parasite-specific targets suitable for further drug or vaccine development.

Assess the capacity of these targets to block transmission of a rodent malaria parasite and report the resulting positive or negative data.

Validate mechanisms of action through targeted genetic modification of candidate genes in transgenic (genetically altered) parasites, enabling direct functional testing.

Prioritise and select a subset of the most promising candidates (approximately five) for translation into subsequent studies focused on human malaria.

Additional outputs from this work will include multiple peer-reviewed publications and preprints, presentations at national and international scientific conferences, and engagement with the public to communicate the significance and impact of the research.

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

The work proposed under this project licence is intended to contribute to the control and potential elimination of malaria. Malaria continues to place billions of people at risk of infection, resulting in substantial morbidity and mortality each year, and remains a major impediment to socioeconomic development in sub-Saharan Africa. In this context, the development of novel strategies to block malaria transmission represents a critical research objective. Our research group maintains established and ongoing collaborations with non-governmental organisations, clinicians, and field sites, ensuring that our findings directly inform the implementation and optimisation of current and future antimalarial interventions.

Over the next five years, the anticipated benefits of this research include the identification of new molecular targets that could be exploited for the development of antimalarial therapeutics. The outcomes of this work will be disseminated through presentations at scientific conferences and publication in peer-reviewed journals, thereby supporting and advancing research efforts across the malaria research community.

In the short term, the primary benefits will be the identification of key molecules involved in regulating malaria parasite transmission to the mosquito host, alongside the evaluation of potential antimalarial intervention strategies. In the medium to longer term, the ultimate goal is to translate these findings into the development of novel antimalarial therapies suitable for clinical application.

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

1). Dissemination of results by peer-review publication (of both positive and negative data).

2). Presentation at national and international scientific meetings.

3). Collaboration with a range of malaria/vaccine/drug related scientific groups to drive clinical development.

4). Links with specialist field-based labs, funders, clinical sites, and non-governmental organisations, to aid the translation of basic scientific research to a therapeutic product.

5). Multiple outreach/public engagement activities.

Species and numbers of animals expected to be used

- Mice: 9000

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.

Rodent malaria parasites Plasmodium berghei and yoelii (P. berghei and P. yoelii) are one of few available safe (non-human infectious), versatile, biologically relevant and reliable species to study transmission of malaria from vertebrate to insect host and back again. The adult laboratory mouse is the most biologically translatable, predictable, and widely accepted host for rearing P. berghei and P. yoelii.

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

Typically, the majority of animals within this project will receive an injection of a drug called phenylhydrazine into the abdomen, with subsequent infection with P. berghei or yoelii 3 days later. At the dosages used, phenylhydrazine induces the production of cells called reticulocytes, which Plasmodium preferentially invades. Thus, the use of phenylhydrazine results in quick and predictable infection, performed by injection into the abdomen or blood vessel.

In a number of experiments within this project, animals will be humanely killed following a mosquito feed to infect mosquitoes. During this process, mice will be placed under general anaesthetic, and exposed to female mosquitoes, who take a small blood meal from mice while they are unconcious. In these cases, the mice maybe previously immunised (by injection into the abdomen, blood vessel, muscle, or under the skin), or drug treated (by administration of experimental anti-malarial compounds by oral treatment, or by injection into the abdomen, blood vessel, or muscle) prior to parasitic infection to determine the transmission blocking efficacy of novel anti-malarial agents. Throughout the work within this project, an animal will only undergo one protocol.

Rather than allowing the mouse to wake up following a mosquito feed, some mice will be killed whilst under deep anaesthesia from which they are not allowed to recover. An unconscious/anaesthetised mouse will feel nothing while we remove a large blood sample.

A number of mice described within this project will not be infected with P. berghei or yoelii but will be instead immunised to produce antibodies against proteins derived from the malaria parasite. Typically, these mice will undergo immunisation by injection into the abdomen, blood vessel, muscle, or under the skin. This may be repeated up to a maximum of 3 times per mouse, with 2 week gaps between immunisations. The resulting immune response will be monitored by examining the amount of antibody present in small volumes of blood taken from mice. When the antibody level is sufficient, these mice will be given general anaesthetic and killed by removal of a large blood sample before the animal wakes up. Antibodies will then be purified from the blood and used in the lab.

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

Expected impacts/adverse effects throughout the project can be split into four different classifications:

1). Impact of infection with Plasmodium:

Infection of mice with P. berghei can result in development of cerebral malaria or anaemia in under 1% of cases. This results in clear signs of distress, which include pale skin tone, bristling of hair, reduced mobility, hunched posture, lethargy/weakness, weight loss and respiratory disease. Infection with P. yoelii results in no symptoms, and is naturally cleared by mice over time.

2). Impact following blood sampling:

Adverse effects of blood sampling are very rare and can be short lived (under 1 hour). Localised bruising may occur in this time period.

3). Impact of immunisation:

When immunising mice, transient pain at injection site may occur. Animals will be monitored following immunisation and any animals showing signs of prolonged distress will be killed, though no adverse effects are envisaged from straight DNA or protein/peptide vaccinations. Observations include regular weight monitoring and daily observations looking for signs of ill health. Expected adverse effects at the site of immunisation include granulomatous reactions(local inflammatory growth), swelling and redness with or without loss of hair over the injection site, and rarely ulceration. These will occur in less than 10% of animals.

4). Impact of treatment with anti-malarials:

Adverse effects resulting from treatment with antimalarials are not expected. Animals will however, be monitored for general signs of deviation from normal health.

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 severity: 10%

Moderate severity: 90%

What will happen to animals used in this project?

- Killed

Replacement

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

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

Human malaria is caused by several species of Plasmodium. However, due to practical, ethical, and safety constraints, it is not feasible to study the transmission stages of lethal human malaria parasites, such as Plasmodium falciparum, at appropriate scale and throughput. Consequently, the use of animal models—most commonly mice—remains essential for investigating the complete malaria transmission cycle.

The rodent malaria parasite Plasmodium berghei is one of the few safe, well-established, and biologically relevant model species available for studying malaria transmission and for generating genetically modified parasites. As P. berghei cannot be continuously maintained in tissue culture in the lab, studies are necessarily limited in this regard, and full replacement of the mouse model is currently not possible. Moreover, there are no alternative non-animal systems capable of supporting vaccine or drug sensitivity testing within a physiologically relevant biological context.

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

Use of cultured human malaria parasites (i.e. – P. falciparum or P. vivax) was considered, as was the potential use of enhanced 3D cell culture systems.

The use of non-antibody systems (e.g. lab produced agents that bind to molecules) to target proteins essential for malarial transmission was also considered.

Typically, blood feeding on live animals is required for mosquito colony maintenance. This has been requested in previous project licences, however, we have successfully adapted our mosquito colonies to feeding via an artificial membrane, greatly reducing the number of rodents requested within this project.

Why were they not suitable?

Unfortunately, despite these options, the need to continue experiments with P. berghei or yoelii in mice remains necessary. Transmission stages of malaria are unsafe and challenging when working with lethal human parasites, therefore the use of animals remains the only way to replicate a full malaria transmission cycle. The ability to perform this is essential for the examination of transmission blocking interventions (both vaccines and drugs), particularly the stages that occur after a mosquito take a bloodmeal. In addition, the generation of transgenic parasites in other malarial species is at an efficiency far less that that observed in the P. berghei model.

There is no proven organoid or culture system that facilitates the development or maintenance of the transmissible stages of P. berghei.

The use of non-antibody systems to target transmission-related molecules does not offer characteristics that are suitable for target identification (e.g. clearance rate, binding affinity). Their use would also not result in information regarding the immunogenicity of vaccine candidates.

We additionally continue to explore viable alternatives to replace the experimental procedures requested, by the use of new experimental culture systems on human malaria parasites. We continue to refer to and check the Replacing Animal Research checklist to identify viable alternatives.

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?

This project requires the use of 9000 laboratory mice for:

(a) examination of basic parasitic transmission biology, phenotypic analysis of transgenic parasites and generation of parasite material for anti-malarial transmission blocking assays, immunofluorescent assay and pulldowns. (5000 mice).

(b) Generation of antibody against Plasmodium transmission blocking targets. (1000 mice)

(c) Assessment of efficacy of anti-malarial transmission blocking vaccine candidates. (1000 mice)

(d) Assessment of efficacy of anti-malarial transmission blocking compound candidates. (1000 mice)

(e) construction of transgenic lines of P. berghei (1000 mice)

These figures are estimated based on experimental requirements in well standardised and accepted experimental procedures performed over the last 20 years.

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

The number of animals proposed to be used under this project licence will be reduced by using well-established, robust systems for parasite propagation, generation of transgenic parasites and mosquito infection. The robustness of these systems greatly reduces the number animals used, as nearly 100% of animals become successfully infected upon inoculation. Our long experience (over 20 years) of working with these systems enables us to reduce the number of animals used by careful planning of experiments, using appropriate controls and replicas to avoid unnecessary experimental repetition. Tissue sharing will be performed where appropriate.

For statistical/experimental design, in the vast majority of occasions, direct experimental measurements are not made on animals during the course of the procedure; instead, animals are used to generate parasite material for subsequent analysis. In these cases, the minimum number of animals

needed to produce this material is used based on the acceptable minimum standards for publication of the results.

If statistical analysis is needed, the numbers of samples/experiments/observations is determined by previous experience, hundreds of available literature and using the most appropriate statistics. In all cases, all experiments are designed to minimize the number of animals used, e.g. data from initial experiments is examined, and only if justifiable the full experiment is required to be carried out (e.g. duplicate and triplicate). For assessment of transmission in the mosquito, specialist statistical analysis is typically required due to non-normal distribution of Plasmodium transmission. All analysis will be performed taking into consideration variation between individual animals and the distribution of the parasite in the mosquito. We will additionally utilise in-house enhanced statistical methods to assess parasite distribution/transmission in mosquitoes, ensuring appropriate design of experiments. During experiments, mice will not be randomised or blinded, however, post-mosquito feed, mosquito populations will be randomised and blinded by a third party, and only unblinded following determination of parasite burden in individual mosquitoes.

In addition to more specialist analysis, the NC3Rs' Experimental Design Assistant has been utilised, along with PREPARE guidelines, and the field accepted-mimima for statistical significance

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

In all cases, all experiments are designed to minimize the number of animals used, e.g. data from initial pilot experiments is examined, and only if justifiable the full experiment is carried out (e.g. duplicate and triplicate). For assessment of transmission, mosquitoes will be examined for intensity of infection and prevalence. Wherever possible, tissue-sharing of animals will be performed.

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 use rodents infected with Plasmodium berghei to examine malarial transmission to mosquitoes.

In almost all our work, the infecting parasite dose is large (to ensure rapid establishment of infection), animals are monitored daily and are used within a few days of infection. The use of phenylhydrazine enables rapid establishment of the required parasite levels before clinical signs. Under these conditions, infections are well tolerated, causing little discomfort to mice with animals typically displaying a normal behaviour. In procedures outlined here, mice are typically humanely killed prior to the start of chronic infection, reducing the severity of any procedures. During generation of transgenic

parasites, drug treatment follows well-documented regimens and is known not to induce adverse phenomena.

During antibody production, resulting immune responses are monitored by taking very small blood samples by the least invasive method. Different immunization programs are established to raise robust immune responses, ensuring maximum chance of success using the smallest number of animals under minimal duress.

For the study of potential anti-malarial drugs, animals will be given doses based on data derived from lab-based studies, and initial doses used are not anticipated to cause health problems since compounds have been tested for toxicity. Known anti-malarials, which are investigated for their effects on transmission or the parasite in the liver, will be used at doses equivalent to those already routinely used for humans and are unlikely to result in toxicity effects.

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

Less-sentient or more immature animals (under 4 weeks old) cannot cannot support the development of a malarial infection or allow the transmission or malaria to or from a vertebrate host. Malaria parasites require mature erythrocytes with specific membrane proteins. The use of immature RBCs prevents parasite invasion, intracellular development and shortens parasite survival time.

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

To improve the quality of life of the animals we will:

i) Reduce contingent harm by using group housing where possible and not using the single housing of mice. This in turn reduces any stress and stereotypical behaviour.

ii). Use environmental enrichment (EE), within what is available to us at our animal facility. In general, EE is an animal housing technique composed of increased space, physical activity, and social interactions, which in turn increases sensory, cognitive, motor, and social stimulation. Igloos, running wheels, saucer wheels, and other objects in the housing environment promote exploration and interaction. EE can be maintained through restraining (e.g. use of restraint tubes whilst handling), thus minimizing stress when for example an injection is needed.

iii). Keep animal transportation to a minimum.

iv). Use specialised scoring sheets to monitor the health of animals undergoing procedures.

v). Utilise non-aversive handling methods, such as cupping or tunnel handling.

vi). For any methods using anaesthesia, mice will be monitored closely for the duration of their recovery.

vii). If required, following veterinary advice, palatable pain medication will be used to reduce discomfort following the formation of local inflammation post vaccination.

viii). If more than 30 bites are given, mice are treated with saline solutions (under the skin) to compensate for blood loss.

ix). Eye drops administered to prevent dry eyes while the animal has no blink reflex while under anaesthesia.

x). Heating pads to ensure maintenance of body temperature.

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

We will use guidelines from the Laboratory Animal Science Association (LASA) to make sure all experiments are conducted appropriately. In particular we will follow the information described in 'Avoiding Mortality in Animal Research and Testing'.

To ensure refined experimental design we will follow the PREPARE guidelines for planning experiments, and for thorough, responsible reporting of results we will follow the ARRIVE guidelines.

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

We constantly review methods for replacement, reduction and refinement provided by the scientific literature and our support team within the facility, attending workshops to stay up to date.

During this project we will continue to be informed about the 3Rs by regularly checking our institute's 3Rs search page, and being registered for regular NC3Rs emails and newsletter updates. Regular reference to guidance documentation provided by the Laboratory Animal Science Association (LASA) and the RSPCA will be made.

We will also ensure continued contact with the organisational teams in the facilities where our animal work will be conducted. Any new recommendations will be incorporated into our experimental plans wherever possible.