



Home Office

## NON-TECHNICAL SUMMARY

# Developing 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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

### Key words

Malaria, Plasmodium, Transmission, Mosquito, Vaccine

### Animal types

Mice

### Life stages

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 and develop new ways to treat or prevent malaria transmission and 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 contribute to the control and elimination of malaria. With 3.3 billion people at risk of infection and 229 million people infected with malaria every year, resulting in over 409,000 annual deaths (WHO World Malaria report 2020). The development of novel anti-malaria blocking strategies is a major technological drive, specifically requested by funders and policy makers, to complement the status of currently utilised anti-malarial interventions.

By focussing on the manner in which the Plasmodium parasite (the causative agent of malaria) passes between mosquitoes and humans, this project aims to find new vaccine candidates or drug targets within the sexual stages of the parasitic lifecycle. Targeting the parasite in this way will arrest the completion of the Plasmodium lifecycle by preventing transmission, thus preventing disease. In addition, the project includes the study of the fundamental biology of the malaria parasite, performed to advance our general understanding of this globally important disease.

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

Based on our key collaborations with pharmaceutical companies, understanding of the parasitic biology as well as previous experience, working under multiple projects we are confident we will be able to achieve success within these broad aims of the proposal. Specifically, aim to:

- Identified ~15 several specific parasite targets for further drug or vaccine development;
- Explore the ability of these targets to block the transmission of a rodent malaria parasite.
- Validate modes of action by modifying targets in transgenic parasites to specifically test these ideas.
- Identified multiple (approximately 5) of the most efficacious candidates to translate to human studies in subsequent work.

Further outputs will include; multiple peer-reviewed publications and pre-prints, presentation at national and international conferences, and public engagement activities.

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

The work proposed to be performed under this project licence aims to contribute to the control and possible elimination of malaria. With billions of people at risk of infection, causing substantial disease and death annually, the disease also presents a significant barrier to socioeconomic development in Sub-Saharan Africa. As such, the development of new anti-malaria blocking strategies is a major objective. Our group has crucial ongoing links with non-governmental organisations, clinicians and field sites, and our research is used to inform the use of current and future anti-malarial interventions.

Specifically, the benefits of our research within the next five years will be to identify new targets which could be used to generate anti-malarial therapeutics. The results of this work will be disseminated via presentation at conferences and in scientific journals to aid others in the field of malaria research.

In the short term, the benefits of this work will be limited to the identification of key molecules that control how malaria parasites transmission to the mosquito host, and the identification of anti-malarial interventions. In the medium to longer term, the development of novel anti-malarials in a clinical settings is the desired goal.

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

- 1). Dissemination of results by 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.
- 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: 11500

## **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 rodent malaria parasite *Plasmodium berghei* (*P. berghei*) is 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 convenient and widely accepted host for rearing *P. berghei*.

### **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 phenylhydrazine into the abdomen, with subsequent infection with *Plasmodium berghei* 3 days later. At the dosages used, phenylhydrazine induces the production of cells called reticulocytes, which *Plasmodium berghei* preferentially invades. Infection will occur 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. 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 procedure.

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 *Plasmodium berghei* 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. 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 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 berghei*:

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 pallor, bristling of hair, reduced mobility, hunched posture, lethargy/weakness, weight loss and respiratory disease.

#### 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, swelling and redness with or without loss of hair over the injection site, and rarely ulceration. These will occur in <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: 17%

Moderate severity: 83%

**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?**

Human malaria can be caused by several species of Plasmodium. However, due to practical, ethical and safety restrictions, transmission stages of malaria are almost impossible to use when working with lethal human parasites, such as Plasmodium falciparum. Therefore, the use of animals, such as mice, are currently the only way to study the full malaria transmission cycle of malaria.

The rodent malaria parasite Plasmodium berghei is one of few available safe, relevant and reliable species to study the transmission of malaria and generate genetically altered parasites. Plasmodium berghei cannot be grown in culture, offering only limited insights into the biology of the parasite. As such, the full replacement of the mouse model is not currently possible. Additionally, there is currently no alternative to animal species that can be used to test with regards to vaccine or drug sensitivity studies in a biologically relevant environment.

## **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.

## **Why were they not suitable?**

Unfortunately, despite these options, the need to continue experiments with *P. berghei* 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). In addition, the generation of transgenic parasites in other malarial species is at an efficiency far less than that observed in the *P. berghei* model.

There is no 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.

## **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?**

The number of animals proposed to be used under this project licence will be reduced by using well-established, robust systems for parasite propagation, transgenesis 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 15 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.

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.

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

For statistical/experimental design, in the 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 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.

In addition to more specialist analysis, the NC3Rs' Experimental Design Assistant has been utilised, along with PREPARE guidelines, and the field accepted-minima 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 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 animals cannot support a malaria infection.

### **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, tube mazes, and other objects in the housing environment promote exploration and interaction. EE can be maintained through restraining (e.g. tube maze whilst handling), thus minimizing stress when for example an injection is needed.
- iii). Keep animal transportation to a minimum.
- iv). Use scoring sheets to monitor the health of animals undergoing procedures.
- v). Utilise non-tail handling methods.
- 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 50 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.

xi). A specialised Malaria Rodent Endpoint score sheet to help with the monitoring of our animals.

**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.