Study Title: A clinical study to assess the safety and feasibility of relapsing *P. vivax* controlled human malaria infection through experimental sporozoite infection of healthy malaria-naïve UK adults, and to characterise parasite growth and immune responses to primary and relapsing *P. vivax* infection

Short title: BIO-006

Ethics Ref: 24/SC/0355

IRAS Project ID: 342867

Date and Version No: 27 Nov 2024 v1.1

Chief Investigator:	Professor Angela Minassian
Investigators:	Professor Simon J Draper
Sponsor:	University of Oxford Research Governance, Ethics & Assurance Team (RGEA) Boundary Brook House, Churchill Drive, Headington, Oxford, OX3 7GB
Funder:	Innovate UK – Horizon Europe Consortium (OptiViVax)

Chief Investigator Signature:

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

Investigator Agreement

"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Council for Harmonisation Tripartite Guideline on Good Clinical Practice."

Professor Angela Minassian		10/10/2024
Chief Investigator	Investigator Signature	Date

Conflict of Interest

1. "According to the Declaration of Helsinki, 2013, I have read this protocol, and declare no conflict of interest"

Professor Angela Minassian

10/10/2024

Chief Investigator	Investigator Signature	Date

2. "According to the Declaration of Helsinki, 2013, I have read this protocol, and declare no conflict of interest"

Professor Simon Draper 10/10/2024

Lead Scientific Investigator	Investigator Signature	Date

MODIFICATION HISTORY

Version	Date	Amendment No.	Author(s)	Modifications
1.0		-	Andrew Duncan Angela Minassian	Created

TABLE OF CONTENTS

1. KEY CONTACTS

Chief Investigator	Professor Angela M Minassian Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE angela.minassian@bioch.ox.ac.uk
Lead Scientific Investigator	Professor Simon J Draper Dorothy Crowfoot Hodgkin Building, University of Oxford, Oxford, OX1 3QU simon.draper@paediatrics.ox.ac.uk
Study Site (1)	Oxford Vaccine Group (OVG) University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE PI at site: Prof Angela Minassian
Study Site (2) (non-recruiting site)	Oxford University Hospitals NHS Foundation Trust John Radcliffe Hospital, Oxford OX3 9DU Churchill Hospital, Oxford, OX3 7LE PI at site: Prof Angela Minassian
Sprozozoite CHMI Unit	Radboud University Medical Center (Radboudmc) Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands
Sponsor	University of Oxford Research Governance, Ethics and Assurance Team, Research Services, University of Oxford Joint Research Office, Boundary Brook House Churchill Drive, Headington, OX3 7GB Tel: 01865 616480 rgea.sponsor@admin.ox.ac.uk
Clinical Trial Unit	Oxford Vaccine Group, University of Oxford, Centre of Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE United Kingdom
Independent Monitor	OVG Monitor Oxford Vaccine Group University of Oxford Centre of Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE

	United Kingdom
Funder(s)	Innovate UK – Horizon Europe Consortium (OptiVivax)
Data Safety Monitoring Committees (DSMC)	Chair & Local Safety Monitor: Dr Ruth Payne, University of Sheffield, United Kingdom Biostatistician: Dr Johannes Mischlinger, Bernhand Nocht Institute for Tropical Medicine, Hamburg, Germany Committee Member: Dr Quirijn de Mast, Radboud University Medical Center, Nijmegen, The Netherlands

2. LAY SUMMARY

Malaria is an illness caused by a parasite infection transmitted by mosquito. The *Plasmodium vivax* species of malaria parasite can hide in the liver in a "dormant" inactive form which can later reactivate to cause a "relapse infection". Without proper treatment, relapse infections can occur several times over the months (or even years) after the initial infectious mosquito bite.

We are conducting a study called BIO-006 to try and find out more about these relapse malaria infections. It is a "malaria challenge study" which means it involves deliberately infecting trial participants with malaria in order to study the disease closely in a controlled research setting.

Up to five healthy adult participants will be bitten by mosquitoes carrying the *Plasmodium vivax* parasite on a designated day at Radboud University Medical Center (RUMC) in Nijmegen, Netherlands. This will require a short trip (approximately 2 nights) to the Netherlands organised and accompanied by the University of Oxford study team. All other appointments will be conducted at the Centre for Clinical Vaccinology and Tropical Medicine in Oxford. Participants will be contacted daily by telephone for the first 6 days after the malaria challenge. From day 7, participants will be seen in person daily at the research clinic by the study doctors. At these visits we will take a small blood sample to assess the parasite growth.

Once we detect the malaria parasites in a participant's blood tests, they will be treated with standard effective malaria tablets (called Malarone) to clear the parasites in blood. Participants may experience some mild symptoms of malaria infection, such as fever and headache, but we will be treating our participants early in the infection to minimise the duration and severity of these symptoms. If for some reason we do not detect any parasites on the daily blood tests, we will treat the participant once they reach day 21 following the mosquito bites.

It is important to note that Malarone will not clear any parasites that are lying dormant in the liver. This is deliberate as we want to study relapsing malaria infections over the following 6-month period. We will monitor participants closely and they will be required to attend a fortnightly in-person clinic. They will also be able to contact the study doctor at any time (24 hours a day) should they experience any symptoms, such as fever, that may indicate relapse malaria infection. When we see the participants, either at the routine clinic or because they have symptoms, we will take a blood sample to assess for any malaria parasites. If we detect any, the participant will be treated as before to clear the parasites from the blood (with Malarone) and return to the fortnightly clinic.

At the end of the 6-month follow-up period, all participants will receive another full course of Malarone and an additional course of a drug called Primaquine that specifically clears any remaining "inactive" parasites from the liver. Primaquine prevents future relapse infections from occurring. After this, email follow-up will continue for all participants for five years to ensure they have not experienced any unexpected relapse infections.

This study will provide us with information about relapse malaria infections (e.g. how often they occur and how the immune system responds and adapts). It is also a proof-of-concept study, meaning that, although we have safely given malaria by mosquito bite to participants in previous studies, we have never allowed participants to experience relapsing infections following a malaria challenge. Success

would mean that we could repeat a similar study in the future (in the knowledge that it works) to test new vaccines or medications that could be used to treat or prevent malaria relapsing infections.

3. SYNOPSIS

<table>
<tr><td>Study Title</td><td>A clinical study to assess the safety and feasibility of relapsing P. vivax controlled human malaria infection through experimental sporozoite infection of healthy malaria-naïve UK adults, and to characterise parasite growth and immune responses to primary and relapsing P. vivax infection</td></tr>
<tr><td>Lay Title</td><td>Development of a relapsing Plasmodium vivax Controlled Human Malaria Infection model</td></tr>
<tr><td>Internal ref. no.</td><td>BIO-006</td></tr>
<tr><td>Study registration</td><td>TBC</td></tr>
<tr><td>Sponsor</td><td>University of Oxford</td></tr>
<tr><td>Funder</td><td>Innovate UK – Horizon Europe Consortium (OptiVivax)</td></tr>
<tr><td>Study Design</td><td>Open-label pilot sporozoite-challenge clinical study with the aim of assessing the safety and feasibility of controlled primary and relapsing human P. vivax malaria infection with the PvW1 clone in five healthy volunteers.</td></tr>
<tr><td>Study Participants</td><td>Healthy adults aged 18-45 years. No previous malaria exposure; Duffy-positive; G6PD normal; CYP2D6 genotype high-metabolisers of Primaquine</td></tr>
<tr><td>Sample Size</td><td>Total: N= up to 5 participants (+2 back up participants)</td></tr>
<tr><td>Planned Study Period</td><td>Total in-person follow-up 7.5 months then fortnightly email until end of year 1. Annual email follow-up thereafter until year 5.
<table>
<tr><th>N=5</th><th>Month 0</th><th>Month 1 – Month 6.5</th><th>Month 6.5</th><th>Month 7.5</th><th>Month 8 – Month 12</th><th>Year 2 – Year 5</th></tr>
<tr><td></td><td colspan="4">In-person</td><td colspan="2">Remote</td></tr>
<tr><td>Study event</td><td>Sporozoite P. vivax CHMI</td><td>Relapse follow-up period</td><td>Definitive P. vivax treatment</td><td>Last in-person visit</td><td>Fortnightly email</td><td>Annual email</td></tr>
<tr><td>P. vivax infection</td><td>Primary P. vivax infection</td><td>Relapse P. vivax infection(s)</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Drug treatment</td><td>Malarone only</td><td>Malarone only</td><td>Malarone and Primaquine</td><td></td><td>-</td><td>-</td></tr>
</table></td></tr>
<tr><td></td><td>Objectives</td><td>Outcome Measures</td></tr>
<tr><td>Primary objective</td><td>To assess the safety, feasibility, and frequency of relapsing P. vivax PvW1 infection after experimental sporozoite-administered Controlled Human Malaria Infection (CHMI)</td><td>i) Safety of primary and relapsing P. vivax infection following sporozoite-administered CHMI as measured by (S)AE occurrences
ii) Success of primary P. vivax infection following sporozoite-administered CHMI as measured by detectable parasitaemia by qPCR +/- clinical symptoms
iii) Frequency of P. vivax relapse infections as measured by number of malaria episodes confirmed by qPCR occurring within a 6-month follow-up period after treatment of primary infection, and time to relapse</td></tr>
</table>

Secondary objective	To assess the immune response to primary and relapsing *P. vivax* PvW1 infection	i) Serological response to a panel of *P. vivax* antigens by ELISA
Exploratory objectives	To assess the infectivity/transmissibility of primary and relapse *P. vivax* PvW1 infection following sporozoite-administered CHMI To further assess the immune response to primary and relapsing *P. vivax* PvW1 infection	i) A qPCR assay will be used to assess gametocyte induction following primary and each relapse *P. vivax* infection i) Serological response by antigen array ii) Cellular responses by flow cytometry iii) Purified total IgG will be screened for functional anti-parasitic activity using growth inhibition assays.
Intervention(s)	Sporozoite Controlled Human Malaria Infection (CHMI) delivered by mosquito bite	
Comparator	None	

4. ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
BMI	Body mass index
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CHMI	Controlled Human Malaria Infection
CI	Chief Investigator
CMV	Cytomegalovirus
CRF	Case Report Form
CYP2D6	Cytochrome P-450 isoenzyme 2D6
DNA	Deoxyribonucleic acid
DSMC	Data Safety Monitoring Committee
EBV	Epstein Barr virus
ELISA	Enzyme linked immunosorbent assay
G6PD	Glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
GP	General Practitioner
HCG	Human Chorionic Gonadotrophin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HRA	Health Research Authority
ICF	Informed Consent Form
LSM	Local Safety Monitor
MAAE	Medically-Attended Adverse Event
NHS	National Health Service
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
qPCR	Quantitative Polymerase Chain Reaction
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RES	Research Ethics Service
RGEA	Research Governance, Ethics and Assurance (formerly Clinical Trials and Research Governance)
RUMC	Radboud University Medical Center
SAE	Serious Adverse Event

SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
WHO	World Health Organization

5. BACKGROUND AND RATIONALE

5.1. Epidemiology of *Plasmodium vivax*

Malaria, the mosquito-borne disease caused by the protozoan parasite *Plasmodium*, continues to cause morbidity and mortality on a global scale despite public health control measures and several recent advances. *Plasmodium vivax* is the most widespread of all the *Plasmodium* species known to cause malaria in humans, with approximately 3.3. billion people living within its geographical distribution (Figure 1).(1) Although *P. falciparum* is more common and more deadly, accounting for an estimated 98% of global malaria incidence, *P. vivax* is not "uncommon" and contributes its own significant burden of disease.(2)

Between 2018-2022, at least 5 million *P. vivax* infections occurred each year.(2) In 2022, *P. vivax* caused approximately half (46%) of the 5.2 million cases of malaria in South East Asia, 29.4% of the 8.3 million malaria cases in the Eastern Mediterranean region (mainly Afghanistan and Pakistan) and most of the malaria cases in the Americas (72% of 552,000 cases).(2)

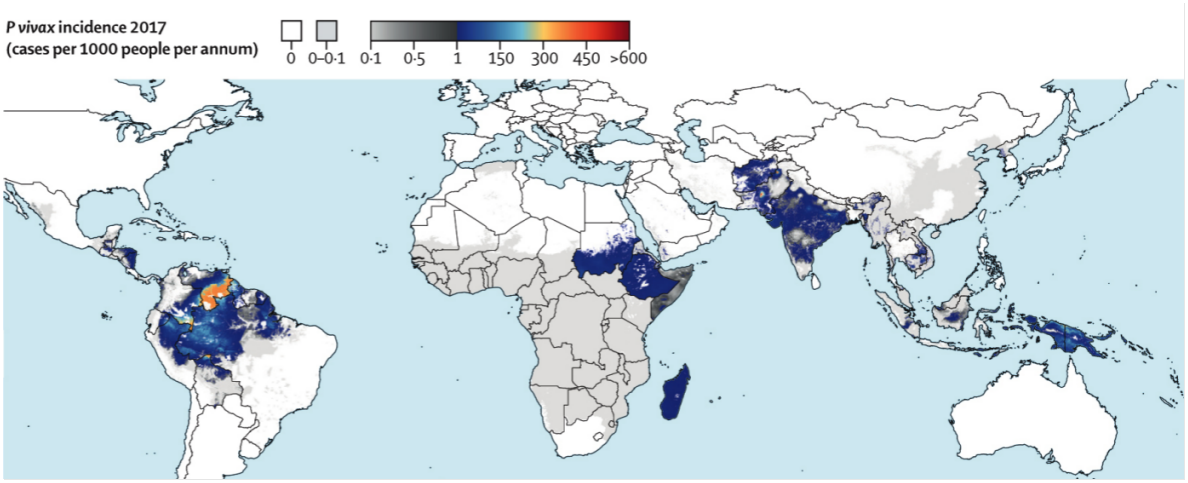


Figure 1: *P. vivax* geographical distribution and incidence (1)

5.2. Lifecycle of *P. vivax*

The lifecycle of *P. vivax* (Figure 2) demonstrates important biological features which set it apart from other *Plasmodium* species.(3) Following the introduction of sporozoites from the bite of infectious female *Anopheles* mosquito, the parasite develops, within the liver, into either an actively dividing schizont or a small dormant form of the parasite, termed a hypnozoite. After a period of replication termed schizogony, schizonts release merozoites which in turn cause the blood-stage infection responsible for clinical disease. Hypnozoites can reactivate to produce an actively dividing schizont weeks to years after the initial inoculation, leading to a recurrence of blood-stage infection temporally

distant from the initial primary infection. These episodes can occur on multiple occasions and are termed relapse infections.

The mechanisms responsible for the reactivation of hypnozoites are poorly understood. However, it is thought that there may be a survival advantage to relapsing infection, ensuring transmission and propagation of the parasite continues in seasonally variable conditions.(3) The formation of hypnozoites is also a feature of the life cycle of *P. ovale* and *P. cynomolgi*, which cause infection in humans and non-human primates respectively.(4) Other noteworthy components of the *P. vivax* life cycle include the early appearance of round gametocytes in the peripheral blood, a predilection of merozoites for reticulocytes as host cells, dependency on the Duffy antigen for infection, circulation of all blood-stage developmental forms in the peripheral blood, and an amoeboid appearance of mature trophozoites.(3)

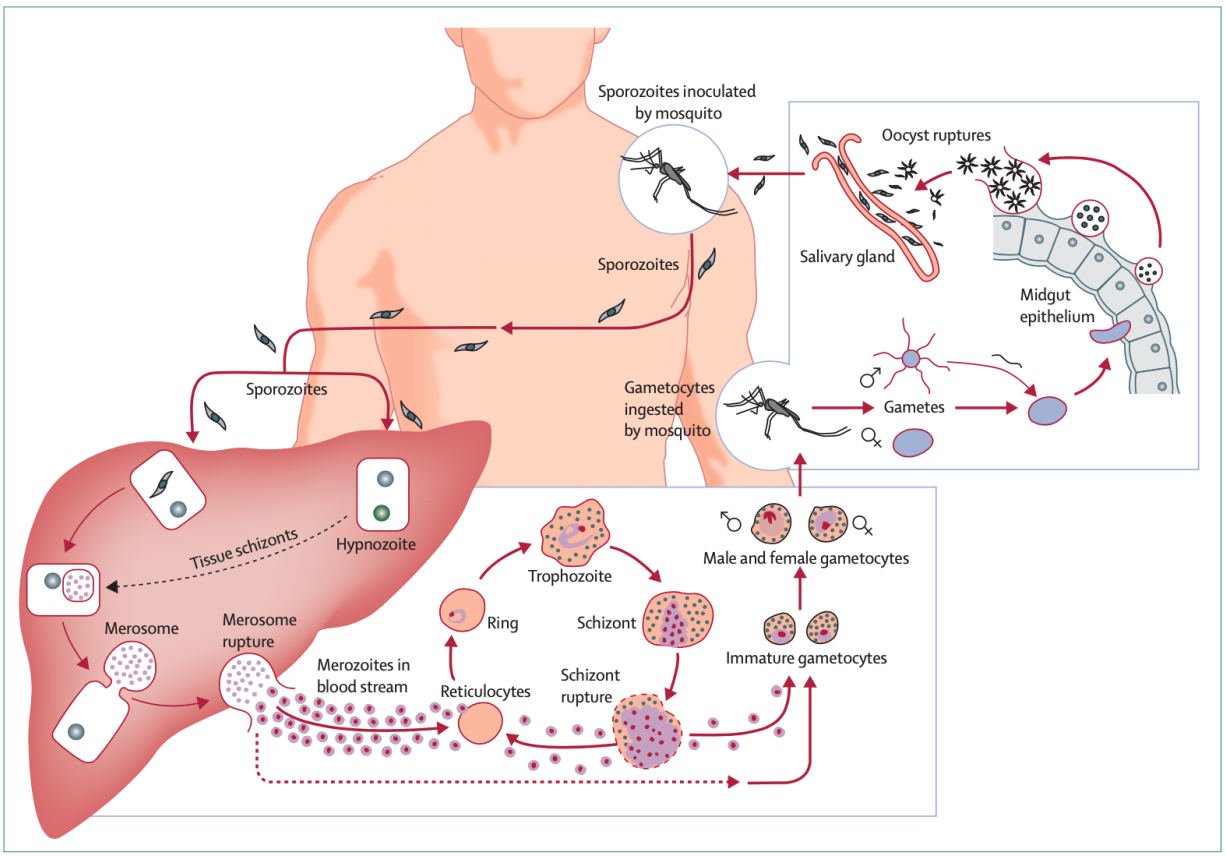


Figure 2: Lifecycle of *P. vivax* (3)

5.3. Implications of *P. vivax* infection

P. vivax causes a febrile illness associated with headache, myalgia, and malaise. Although these symptoms are non-specific, high fever and rigors are more common in *P. vivax* than *P. falciparum* malaria due to the synchronicity of schizont rupture. The classic paroxysms of fever last 4-8 hours and occur every 48 hours once the parasite is established in asexual reproduction cycles.(5)

P. vivax was previously considered a "benign" malaria disease, particularly compared to *P. falciparum,* but this view is no longer accurate.(6) *P. vivax* can cause severe malaria disease (as defined by World Health Organisation (WHO) criteria(7)) and can be fatal in some cases. In a retrospective review of

hospital admissions in Northeastern Indonesian Papua, 10% of the 88 malaria-attributed deaths occurred in patients with *P. vivax* infection.(8) Similarly, in children hospitalised with malaria under the age of 5 years in Papua New Guinea, 8.8% of the 978 cases of *P. vivax* demonstrated clinical features of severe malaria, compared to 11.7% of the 2223 cases of *P. falciparum*.(9) In pregnancy, *P. vivax* malaria is associated with maternal anaemia and low birth weight,(10) while a single episode of *P. vivax* malaria in the first trimester is associated with miscarriage.(11)

Due to the relapsing nature of the infection, the consequences of *P. vivax* infection extend beyond the initial primary infection. An individual with *P. vivax* infection may experience up to 10-30 relapse episodes over the course of childhood and working life, each associated with a period of illness, leading to morbidity, an adverse impact on school performance, loss of earnings and additional household costs.(12, 13) The overall global cost of *P. vivax* infection to the individual in terms of lost productivity, healthcare costs and transport to clinics has been estimated to be in excess of $1 billion per year.(5)

5.4. Relapsing *P. vivax* infection

Recurrent clinical episodes of *P. vivax* malaria may be due to one of the following:(14)

- Reinfection: New infection following exposure to another infected mosquito
- Recrudescence: Failure to clear blood-stage infection due to ineffective or incomplete treatment of primary infection
- Relapse: Reactivation of dormant liver hypnozoites weeks to years after the initial infection to cause blood-stage infection

Clinically distinguishing these different sources of infection is almost impossible. Nevertheless, several previous studies have attempted to quantify the contribution of relapse infections to the incidence of *P. vivax* malaria.

Adekunle *et al.* propose, based on mathematical modelling, that up to 96% of *P. vivax* infections in certain areas in Thailand are due to hypnozoite reactivation.(15) Commons *et al.* performed a review of data from anti-relapse efficacy studies and estimate the proportion of *P. vivax* recurrences due to relapse to be 85%.(14)

Insights into relapsing malaria have also been obtained from observational studies of travellers(16-18) or soldiers(19-21) who have experienced *P. vivax* infection after returning to non-endemic areas following transient exposure in areas of *P. vivax* transmission. Genotyping has demonstrated that as many as 78% of relapse infections are caused by a different *P. vivax* parasite from the initial pre-treatment infection.(16, 18, 20) These heterologous relapses may be due to simultaneous inoculation of sporozoites with multiple *P. vivax* genotypes or reactivation of hypnozoites from an earlier primary infection. This makes it challenging to comment on the true frequency of relapse from a single genotype *P. vivax* infection. Additionally, the latency (time to first relapse) and frequency of relapse infections varies significantly by geographic region.(22)

5.5. Current anti-hypnozoite agents - Primaquine and Tafenoquine

The majority of *P. vivax* incident cases arise from reactivation of dormant hypnozoite, rather than a primary sporozoite infection from the bite of an infectious mosquito. Therefore, therapies effective

against hypnozoites are key in the treatment of *P. vivax* malaria, and prevention of onward transmission.

Until recently, Primaquine was the only available anti-malarial with efficacy against hypnozoite stage of *P. vivax* infection. Despite first being developed in 1946, its mechanism of action remains poorly understood.(23) In recent years, Tafenoquine, another 8-aminoquinoline, has been approved for use in the radical cure of *P. vivax* malaria.(24) Tafenoquine has the advantage of only requiring a single dose compared with up to 14 days of treatment with Primaquine. However, it has not solved all the problems associated with Primaquine treatment and, in a meta-analysis of Phase 3 studies, Tafenoquine was not shown to be non-inferior compared to Primaquine at preventing *P. vivax* recurrence at 6 months.(25) Primaquine and Tafenoquine are both administered shortly following (or overlapping) effective anti-malarial medication against blood-stage infection.(26)

Both Primaquine and Tafenoquine are contraindicated in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, a genetic condition with considerable geographic overlap with *P. vivax* (Figure 3).(27, 28) Treatment in affected individuals risks inducing severe (even fatal) haemolysis. Testing for G6PD deficiency is therefore required prior to treatment with Primaquine or Tafenoquine but, in many endemic regions, it is not readily available.(28) These drugs would therefore not be suitable for mass drug administration. Both Primaquine and Tafenoquine are also contraindicated in pregnancy.(23, 24)

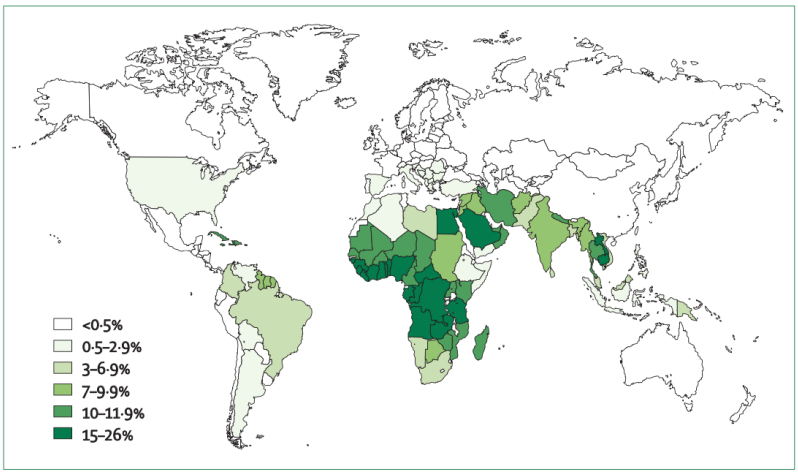


Figure 3: G6PD Deficiency prevalence from Capellini *et al.*(27)

Despite being the only available treatments, Primaquine and Tafenoquine may be only partially effective at clearing the dormant hypnozoites responsible for relapsing infection. In travellers returning to the UK, Doherty *et al.* report 7/39 patients experienced a further infection in the 6 months following Primaquine treatment (15mg daily for 14 days, treatment not observed).(17) In soldiers returning to Australia from deployment in East Timor, 210/5500 experienced *P. vivax* infection following terminal prophylaxis with Primaquine (7.5mg three times daily for 14 days, treatment not observed).(19) Although compliance with the terminal prophylaxis is unclear, 44/210 experienced a further relapse (following another course of Chloroquine and Primaquine 22.5mg or 30mg daily 14 days), 11 had a second relapse (following further treatment) and 2 had a third relapse. As these studies involve the return of individuals to non-endemic areas, it is likely that these recurrences are true

relapse infections following inadequate clearance of liver hypnozoites, either due to poor compliance, suboptimal dosing, or partial efficacy.

Current WHO guidelines recommend either a standard 14-day course of Primaquine 0.25mg/kg/day (total dose 210mg) or considering a shorter 7-day course of high dose Primaquine 0.5mg/kg/day (total dose 210mg) to increase the likelihood of adherence to the full treatment regimen.(7) In the United Kingdom, current standard practice is to treat adults with high dose Primaquine 30mg once daily for 14 days.(26) Nevertheless, a meta-analysis by Commons *et al.* assessing Primaquine dosing regimens demonstrated that 8.1% of patients experience recurrent *P. vivax* infection within the 6 months following high dose Primaquine treatment (7mg/kg total dose, 98% fully supervised treatment).(29) At least a proportion of these cases may be due to reinfection as these individuals resided in areas of *P. vivax* transmission, 88% in areas classified as moderate or high transmission intensity.

It is now recognised that polymorphisms associated with the human cytochrome P-450 isoenzyme 2D6 (CYP2D6) can influence Primaquine metabolism and therefore efficacy of treatment.(30) CYP2D6 is responsible for metabolising Primaquine into active redox metabolites. Bennet *et al.* reported multiple relapses occurring following Primaquine treatment in two individuals who were participants in a sporozoite *P. vivax* controlled human malaria infection study.(31) One of these individuals experienced relapse at 9 and 18 weeks, while the second individual experienced relapse at 11, 20 and 48 weeks following sporozoite infection. After each relapse, parasitaemia was cleared and Primaquine treatment was completed at a total dose of 6 mg/kg. By study completion the participants had been followed up for 5 years and had not had any further relapses. Exploratory genotyping for the cytochrome P450 allele CYP2D6 was undertaken in 25 of the 33 volunteers. The volunteers who experienced relapses despite Primaquine treatment were found to have either an intermediate-metaboliser phenotype or poor-metaboliser phenotype. These phenotypes were associated with significantly lower levels of Primaquine clearance 24 hours after dosing.(31, 32) Nevertheless, Primaquine treatment failure has also been reported in the context of extensive metaboliser phenotype.(16)

5.6. Further challenges

The study of relapsing *P. vivax* malaria (and therefore development of novel anti-hypnozoite agents) is challenging for several reasons.(4) Firstly, a continuous culture of *P. vivax* has never been achieved.(33) Therefore *in vitro* study of liver or blood stage infection requires a supply of infected mosquitoes to provide viable sporozoites. Secondly, a stable, infectable, and long-lasting hepatocyte cell line is required to study hypnozoite formation and activation, and to test potential therapies. Thirdly, as hypnozoites are relatively inert and reside in the liver, they are not amenable to straightforward sampling from infected individuals, and there are currently no known systemic biomarkers of the presence of dormant (or activating) hypnozoites. However, recent progress has been made, with *in vitro* studies achieving successful *in vitro* hypnozoite formation and reactivation for *P. vivax* and *P. cynomolgi*.(34-36)

5.7. Rational for current study

Relapse infections account for most cases of *P. vivax* malaria. The elimination of *P. vivax* hypnozoites is therefore an essential component in the pursuit of malaria eradication. However, the current drugs

available are not fit for this purpose due to contraindications, polymorphisms associated with poor metabolism, and partial efficacy. Novel drug therapies against hypnozoites are required. In addition, *P. vivax* vaccine candidates must be able to demonstrate efficacy in preventing the development of hypnozoites in order to have a meaningful impact on *P. vivax* incidence and onwards transmission. Current *in vitro* strategies for investigating new drug and vaccines are hampered by an inability to produce a long-term culture of *P. vivax* parasite, while "real world" studies of relapsing *P. vivax* disease are confounded by an unquantifiable contribution from primary reinfections and heterologous relapses. We therefore need new controlled research methods to improve our understanding of homologous hypnozoite reactivation, and provide a platform for the development of novel therapies (curative or preventative) against relapsing *P. vivax* malaria.

5.8. Controlled Human Malaria Infection (CHMI) studies

Controlled human infection studies involve the deliberate infection of human participants with an infectious pathogen, often termed a "challenge agent".(37) They allow close examination of the pathogenesis and immunology of disease, as well as assessment of therapeutic or preventative interventions (such as vaccines) in an experimental setting.

Human "challenge studies" have contributed to our understanding of numerous microbial diseases including malaria, influenza, cholera, typhoid and hepatitis.(38) A review by the UK Academy of Medical Sciences recognised that such studies are desirable for providing proof of concept for prophylactic and therapeutic interventions and can significantly accelerate progress to Phase 2 and 3 studies.(37, 38)

P. falciparum and *P. vivax* are particularly well-suited for challenge studies, with short asymptomatic periods, established diagnostic tests (such as microscopy and/or real-time quantitative polymerase chain reaction (qPCR)), and effective treatments. There are no known long-term sequelae following promptly and appropriately treated malaria infection.

Controlled Human Malaria Infection (CHMI) studies can either involve a blood-stage challenge, where a *Plasmodium* infected blood inoculum is introduced by intravenous injection, or a sporozoite challenge from the bite of infectious mosquitoes. For *P. vivax*, a sporozoite challenge would be essential for the study of relapse infection to allow the formation of hypnozoites (Figure 2).

5.9. History of human malaria challenge

Deliberate infection of humans with malaria was first performed as a therapy for patients with neurosyphilis in 1917 by Wagner von Jauregg - an Austrian physician who received the Nobel Prize for this treatment in 1927.(39, 40) The objective was to induce high fever to kill *Treponema pallidum* – the bacterium that causes syphilis. Thousands of patients underwent malariotherapy and were administered malaria parasites by bites of infectious mosquitoes or direct injection of sporozoites or blood-stage parasites. *P. vivax* was preferred over *P. falciparum* because of its low rate of complication, even at high levels of parasitaemia, and the good induction of fever. Malariotherapy provides a wealth of information about *P. falciparum* and *P. vivax* infection, which has been reviewed previously.(40) The practice stopped with the advent of antibiotics effective against syphilis.

Deliberate infection with *P. vivax* was also conducted in the USA from the 1940s to 1970s. These studies mainly examined compounds for their potential use as anti-malarial drugs, but also assessed the ability to immunise participants by exposing them to *P. vivax* infected irradiated mosquitoes, followed by challenge with non-radiated infected *Anopheles*.(41, 42) Similar studies were also carried out at the United States Penitentiary, Atlanta and Maryland, USA.(43) Key discoveries of the biology of *P. vivax* were made during this period, including the association between Duffy negativity and resistance to *P. vivax* infection.(44)

Following the development of protocols for the continuous culture of asexual *P. falciparum* in 1976 (45) and for the generation of mature *P. falciparum* gametocytes *in vitro* in 1981,(46) it became possible to reliably infect laboratory-reared mosquitoes, kick-starting the modern era of controlled human *P. falciparum* infection trials.(47) The first well-documented CHMI study was carried out in 1986 at the US Walter Reed Army Institute of Research, the US Naval Medical Research Institute and the US National Institutes of Health. Six volunteers were infected with *P. falciparum* sporozoites by the bites of infectious laboratory-reared *Anopheles freeborni* and *Anopheles stephensi* mosquitoes.(48) The following year, the efficacies of the first recombinant protein and synthetic peptide *P. falciparum* vaccines were tested in experimentally infected volunteers.(49, 50)

Since the late 1980s, the number of institutions carrying out CHMI with *P. falciparum* has been growing. These models have significantly contributed to the *P. falciparum* vaccine pipeline.(51, 52)

5.10. Ethical considerations of CHMI trials

Participants in CHMI trials are healthy volunteers who do not obtain direct health benefit from participation. Challenge trial investigators must exercise all possible safeguards for participant safety to ensure that trial participation is of minimal risk. Investigators must also ensure that maximal scientific benefit accrues from each challenge trial. Key ethical considerations agreed by consensus of the field are outlined in a publication by Targett *et al.* from 2013, and include:(53)

1. Participant safety is the paramount consideration in conduct of CHMI trials.
2. Adherence to both international and local guidelines with respect to ethical considerations and in accordance with the Declaration of Helsinki and local regulatory and ethics committee requirements.
3. CHMI trials should be conducted according to International Council for Harmonisation and/or WHO Good Clinical Practice Guidelines with the aim of maximising scientific benefit whilst minimising risk.
4. The raw data (whether microscopy and/or qPCR where available) from challenge trial datasets should be made publicly available to facilitate scientific benefit to the community.

If an unexpected Serious Adverse Event (SAE) which is possibly related to CHMI occurs at a challenge trial centre, recognising legal restrictions, every effort should be made to communicate information on this SAE to the community of challenge trial centres within 90 days of the occurrence of the SAE.(53)

5.11. Standardisation of CHMI studies

Following a collaborative process involving investigators from the US Military Malaria Vaccine Program, Sanaria, University of Maryland, University of Oxford, Radboud University Medical Center (RUMC), The Seattle Biomedical Research Institute and the KEMRI-Wellcome Kilifi Research Programme, a consensus document; *"Standardization of Design and Conduct of P. falciparum Sporozoite Challenge Trials"* was developed and provides a comprehensive guide to the appropriate conduct of sporozoite CHMI studies.(53)

- All participants should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination, pregnancy test for participants of childbearing potential.
- Participants should be questioned about the occurrence of adverse events and use of medication at each follow-up visit.
- In the event that a participant does not attend a scheduled follow-up visit it is imperative that Investigators find that participant as quickly as possible and assess them for patent parasitaemia and clinical malaria. Should the participant withdraw consent from further follow-up prior to receipt of anti-malarial drugs, it may be appropriate to withdraw the participant from the trial protocol and administer a course of anti-malarial chemotherapy under close supervision.
- Grading and reporting of adverse events should be performed using international and local guidelines. It should be noted that the occurrence of a low frequency of grade 3 severe adverse events, of short duration, and with no long-term sequelae, is not unexpected in CHMI studies. A minority of those challenged are known to experience grade 3 systemic adverse events and this fact should be included in the informed consent form.
- Vital signs should be recorded at each visit for medical attention. Directed physical examination should be performed when necessary.
- It is critical that every participant must receive every dose of anti-malarial therapy. In some settings fully directly observed treatment will be essential. Where directly observed treatment is not used, Investigators must follow participants closely to ensure compliance with the treatment regimen.
- After challenge, all participants should be followed until they have completely finished anti-malaria treatment.
- Participants should be evaluated at least two weeks after finishing treatment.

A local safety monitor and an independent safety monitoring committee should be established to act as independent experts in evaluating adverse events. The safety monitor or monitoring committee may advise the Investigators on initiating anti-malarial treatment for a specific participant or participant group. While safety monitoring committees are not a requirement for Phase 1 trials, they should be considered a requirement for CHMI trials which have an efficacy/human challenge component and which have major potential safety concerns.(53)

5.12. Clinical presentation post-CHMI

In 2018, it was estimated that controlled human malaria infections had been conducted globally in over 2,650 participants with *P. falciparum* and 300 participants with *P. vivax*.(54) Based on these data, approximately one-fifth of participants temporarily develop symptoms graded as severe (symptoms that prevent daily activities), but severe or life-threatening malaria has never occurred.(55) The

expected symptoms, time-course and management of clinical malaria are outlined in Section 5.19.5, Section 9 and Section 11.2.

5.13. Laboratory abnormalities including transiently raised liver function tests

Routine laboratory checks generally show a moderate decrease in leukocyte and platelet numbers during infection, with no change in haemoglobin concentration.(56) Bleeding or thrombogenic complications have never been described.(55, 56) Abnormalities of liver enzymes have been observed, but these abnormalities have rarely resulted in clinical manifestations (just one participant with raised Alanine transaminase (ALT) associated with abdominal pain and vomiting in a *P. vivax* study from Cali, Colombia (57)) and they resolved after a few days.

A recent meta-analysis, which analysed and compared patients with uncomplicated imported *P. falciparum* malaria and participants who had undergone CHMI, found that liver function test abnormalities were common in *P. falciparum* malaria. Liver enzyme elevations were observed in 69% of patients with uncomplicated imported malaria and 87% of patients with severe disease. In CHMI studies, liver enzyme elevations were reported in 52% of the cases.(58)

While a clear explanation for the observed abnormalities is lacking, they have been linked to parasitic load. Indeed, a retrospective analysis of CHMIs conducted at the RUMC, showed that participants treated based on a qPCR threshold of 100 *Pf*/mL had a lower percentage and severity of liver function abnormalities compared to those treated at higher thresholds (personal communication). Importantly, only participants undergoing their first in life infection display severe or moderate adverse events related to elevated alanine aminotransferase while in 75% of reinfected participants liver enzymes remained within normal range and 25% show only a slight elevation classed as a mild adverse event. All abnormalities were transient and resolved spontaneously within 3 to 6 weeks. Data from the VAC063 study (ClinicalTrials.gov Identifier: NCT02927145) compared to the findings from a meta-analysis by Reuling *et al.* showed that participants who underwent three successive *P. falciparum* challenges had a reduced severity of liver enzyme abnormalities after their third challenge compared to malaria-naïve participants infected with malaria for the first time, despite the peak parasite density being higher among reinfected participants.(59, 60) It is possible that the combination of other risk factors, such as use of paracetamol and/or individual susceptibility, may also have triggered liver enzyme elevations. Recent recommendations have been made to minimise hepatotoxicity risk in CHMI study participants.(61)

In our Oxford CHMI studies to-date, any abnormalities in liver function tests have also been transient and have resolved spontaneously. In order to minimise the risks for participants , we have also implemented the following criteria during the malaria challenge period:

1. Regular safety monitoring to assess asymptomatic liver function test abnormalities including several time points after anti-malarial drug treatment.
2. Avoiding additional triggers that may cause elevations in liver enzymes, including alcohol, during CHMI period (until completion of Malarone or Riamet treatment) or during any relapse malaria infections (until completion of Malarone or Riamet treatment).
3. A maximum dose of 3000 mg per day of paracetamol/acetaminophen.

5.14. Cardiac Adverse Events

Over the past twenty years, five cardiac AEs have been reported after CHMI with the *Pf*NF54 strain (parental strain of the 3D7 clone). All have occurred in the Netherlands and all resolved without any apparent sequelae.

The first one dates back to 2002 and occurred at the RUMC in The Netherlands. A 39-year old male participant in a CHMI study, who was retrospectively discovered to have had a pre-existing stenosis of the ramus circumflexus, developed symptoms and signs of myocardial infarction 11 days after challenge with two *Pf*NF54-infected mosquito bites and one day after commencing treatment with chloroquine upon development of a positive thick blood smear.

In 2007, a 20-year old, otherwise healthy, female participant, who had participated in a Phase 1/2 clinical trial with the anti-malarial vaccine candidate *Pf*LSA-3-rec adjuvanted with aluminium hydroxide and had subsequently been exposed to wild-type parasites for CHMI, developed acute retrosternal pain two days following treatment with artemether/lumefantrine. She was diagnosed as probable myopericarditis, although ischaemia could not be ruled out. Although a definite relationship between the cardiac event, which resolved fully and rapidly, and the experimental malaria infection was not established,(62) it has been generally agreed that participants with an increased risk of cardiac disease should be excluded from such trials.(53) Follow-up was unremarkable.

A further case of myopericarditis was identified in 2013 in a *P. falciparum* CHMI study, also at the Nijmegen centre, Netherlands, but in this case the individual was also diagnosed with an intercurrent rhinovirus infection so the relation to malaria infection is again uncertain. There was a brief episode of clinical chest pain and the participant made a full recovery.(63)

In November 2014 a 23-year old healthy male taking part in the BMGF1 study (NL48301.91.14) experienced a cardiac SAE 10 days after a malaria infection under chloroquine prophylaxis. This SAE concerned an asymptomatic highly sensitive troponin T elevation (maximally 168 ng/l) diagnosed as a mild myocarditis.

Most recently, in October 2020, a fifth cardiac SAE occurred in the context of the CPS135 trial which aims to investigate heterologous protection induced by the CPS immunization approach. A 23-year old woman with no cardiac risk factors or history of cardiac disease, developed retrosternal pain 10 days after the first inoculation with the bites of 15 *Pf*NF135 infected mosquitoes and one day after completion of treatment with artemether/lumefantrine. She had elevated cardiac biomarkers, with troponin T reaching 331 ng/L and creatinine kinase 230 U/L, and minimal dynamic ECG changes. The participant received no therapeutic intervention other than a single dose of metoprolol prior to coronary CT-A and repeated sublingual nitro-glycerine. During admission her episodes of chest pain spontaneously subsided and the cardiac biomarkers normalised. The cardiac MRI, coronary CT-A, coronary angiogram and echocardiography showed no evidence of myocarditis, takotsubo or coronary occlusion/dissection and no radiological evidence of myocardial sequelae. In conclusion, the participant presented with acute coronary syndrome with no conclusive findings for the cause and no evident myocardial sequelae (M. McCall, personal communication).

As a result of these cardiac SAEs, safety procedures for CHMI have been progressively intensified. Centres, such as Nijmegen, who previously conducted challenges with the *Pf* NF54 strain, have now

switched to performing challenges with *Pf* 3D7 with which there have been no cardiac SAEs. In Oxford, we have only ever conducted blood-stage *P. falciparum* CHMI studies using the *Pf* 3D7 clone.

No cardiac adverse events have been reported in the context of *P. vivax* CHMI studies. However, we continue to adhere to stringent procedures such as strict exclusion criteria related to cardiac medical history.

5.15. *P. vivax* sporozoite CHMI

P. vivax cannot be cultured long-term *in vitro* and only a few studies have experimentally infected human participants with this parasite. The majority of these studies have been mosquito-bite delivered sporozoite challenge trials, conducted in Cali, Colombia,(64-66) the Walter Reed Army Institute of Research, Maryland, USA (31) and Oxford (VAC068, ClinicalTrials.gov Identifier: NCT03377296)(67). These are summarised below in Table 1 adapted from Payne *et al.*, Trends in Parasitology, 2017 (33)).

Table 1: Sporozoite CHMI studies adapted from Payne *et al.*(33)

Sporozoite P. vivax CHMI study	No. volunteers	Pre-patent period (days)[#]	No. infective bites	No. volunteers with patent parasitaemia
Herrera S *et al.* **Cali, Columbia** Successful sporozoite challenge model in human volunteers with Plasmodium vivax strain derived from human donors.(65)	18	9 – 13	2 - 10	17/18
Herrera S *et al.* **Cali, Columbia** Consistent safety and infectivity in sporozoite challenge model of Plasmodium vivax in malaria-naive human volunteers.(66)	17 Duffy +ve 5 Duffy -ve	9 – 16	2 - 4	17/17 (Duffy +ve) 0/5 (Duffy -ve)
Arevalo-Herrera M *et al.* **Cali, Columbia** Plasmodium vivax sporozoite challenge in malaria-naive and semi-immune Colombian volunteers.(64)	7 malaria-naïve 9 semi-immune	11 – 13	2 - 4	16/16
Arevalo-Herrera M *et al.* **Cali, Columbia** Protective Efficacy of Plasmodium vivax Radiation-Attenuated Sporozoites in Colombian Volunteers: A Randomized Controlled Trial.(57)	12 Duffy +ve vaccinees 2 Duffy +ve controls 5 Duffy -ve controls	12 – 13	2 - 4	7/12 vaccinees 2/2 Duffy +ve controls 0/5 Duffy -ve controls
Arevalo-Herrera M *et al.* **Cali, Columbia** Randomized clinical trial to assess the protective efficacy of a Plasmodium vivax CS synthetic vaccine.(68)	16 malaria-naive 11 vaccinees 5 controls 16 semi-immune 11 vaccinees 5 controls	Malaria-naïve 14-17 Semi-immune 12-19	2-4	12/16 malaria-naive 7/11 vaccinees 5/5 controls 16 semi-immune 8/11 vaccinees 4/5 controls
Bennett JW *et al.* **Walter Reed Army Institute of Research, USA** Phase 1/2a Trial of Plasmodium vivax Malaria Vaccine Candidate VMP001/AS01B in Malaria-Naive Adults: Safety, Immunogenicity, and Efficacy.(31)	27 vaccinees 6 infectivity controls	10 – 13 10 – 11	5	27/27 vaccinees 6/6 controls
Minassian AM *et al.* **Oxford, United Kingdom** Controlled human malaria infection with a clone of Plasmodium vivax with high-quality genome assembly.(67)	2 Duffy +ve	13-14	5	2/2

Pre-patent period refers to period before malaria diagnosis following sporozoite infection

A total of five sporozoite challenge studies have been conducted in Cali, Colombia. The first trial involved eighteen healthy volunteers, exposed to the bites of 2-10 *P. vivax* infected *Anopheles albimanus* mosquitoes, of which seventeen developed infection. The authors speculate that the volunteer who did not develop malaria had surreptitiously taken anti-malarial medication, but this was never confirmed.(65) There were no SAEs in this trial, but seven volunteers required fluid therapy due to nausea and vomiting, and five developed blurred vision lasting 2-3 days after treatment initiation with chloroquine. The second *P. vivax* CHMI trial was carried out by the same group in Colombia, aiming to demonstrate the reproducibility of this method of infection using three different *A. albimanus* mosquito lots fed on blood from three *P. vivax*-infected donors.(66) Seventeen individuals whose red blood cells were positive for the Duffy antigen/chemokine receptor (DARC, "Duffy positive") and five Duffy negative controls were enrolled then randomly assigned to three groups (with six Duffy positive individuals in two of the groups and five in the third group). Following 2-4 bites from infected mosquitoes, all Duffy positive participants (and none of the Duffy negative participants) developed blood-stage malaria. A third *P. vivax* CHMI trial was carried out in Cali among both "semi-immune" (previously-exposed; n=9) and malaria-naïve adult volunteers (n=7).(64) Symptoms were significantly worse among the malaria-naïve subjects but there were no SAEs.

The fourth CHMI trial from the Cali group assessed "immunisation" through repeated exposure to radiation-attenuated sporozoites, delivered by mosquito bite.(57) Moderate efficacy was demonstrated in 42% of volunteers, who were sterilely protected following sporozoite challenge (five out of twelve Duffy positive participants protected). There were no reported SAEs related to immunisation, although one volunteer developed severe elevation of hepatic transaminases (>10 times the upper limit of normal [x ULN]) with associated abdominal pain and vomiting following CHMI, with no alternative cause found. These symptoms resolved spontaneously.

The most recent CHMI trial in Cali was a randomised, double-blind, controlled vaccine clinical trial to assess the safety and protective efficacy of the *P. vivax* circumsporozoite (CSPvCSCS) protein in healthy malaria-naïve (Phase 2a) and semi-immune (Phase 2b) volunteers. Participants (n = 35) were divided into naïve (n = 17) and semi-immune (n = 18) groups and were immunised at months 0, 2, and 6 with PvCS formulated in Montanide ISA-51 adjuvant or placebo (adjuvant alone).(68) Three months after the last immunisation, all participants were subjected to CHMI. All naïve controls became infected but significant parasitaemia reduction, including sterile protection, was observed in several CSP-vaccinated volunteers in the Phase 2a (6/11) (54%, 95% CI 0.25-0.84) and Phase 2b (7/11) (64%, 95% CI 0.35-0.92). However, no difference in parasitaemia was observed between the Phase 2b experimental and control subgroups.

5.16. Oxford experience of *P. vivax* sporozoite CHMI

The first *P. vivax* sporozoite challenge in Europe was conducted in April 2018, by the University of Oxford within the VAC068 study (ClinicalTrials.gov Identifier: NCT03377296).(67) Two malaria-naïve adult participants were exposed to infectious bites by *Anopheles dirus* mosquitoes.

Infection in the mosquitoes was established by direct membrane feeding on the blood from a *P. vivax* infected source patient, in Songkhla, Southern Thailand, before transport of the infected mosquitoes to the UK. The mosquitoes were provided by The Mahidol University, Bangkok. The colony is maintained through feeding on rigorously screened human blood (provided by the Red Cross) to induce egg-stimulation. Animal blood is never used. For infection of mosquitoes, a source patient was recruited from a medical clinic in one of Thailand's Southern endemic areas – Songkhala (~7 hours' drive from the Vivax Research Unit in Bangkok). Microscopic diagnosis of the source patient was established at both the field site and in the Bangkok reference laboratory, where qPCR analysis confirmed *P. vivax* mono-infection.

Molecular speciation by qPCR (research-grade laboratory assay) was verified at the Jenner Institute Laboratories and clonality of the infection was established by the Wellcome Trust Sanger Institute in Cambridge, UK, on a 2mL whole blood sample, sent directly by courier from Thailand to Oxford. Mosquitoes were infected via direct membrane feeding with blood from the source patient, and infectivity was confirmed at 5-7 days post-feeding through oocyst count on dissection of the midgut.

Two healthy UK participants were recruited and exposed to five infectious *Anopheles dirus* mosquito bites, defined as bites by mosquitoes with >10 sporozoites as detected on microscopic examination of salivary glands post-feeding. Both participants were followed up from days 1-6 post-challenge by telephone and from the evening of day 6 at twice-daily clinic visits. Blood sampling was performed at each visit for thick film microscopy and qPCR. Clinical symptoms consistent with malarial infection (including fatigue and subjective feverishness, chills, headache, loss of appetite, nausea, and malaise) were reported from day 11.5 post-challenge in Participant 1 and from day 14 in Participant 2, with both becoming febrile at day 14. Thick film positivity was defined as the detection of at least two morphologically normal malaria parasites seen in 200 high-power (1000x) fields. Thick film microscopy was positive at day 14.5 in Participant 1 and at day 13.5 in Participant 2, corresponding to 31,010 and 16,717 genome copies/mL by qPCR, respectively.

A blood donation was performed in each participant immediately prior to initiation of treatment in the afternoon and evening of day 14. A 250mL blood sample was collected using aseptic technique, via a whole blood donation kit (Leuokotrap WB, Haemonetics Corp), containing an in-line leukodepletion filter. For anonymization of the blood donor, blood collected for cryopreservation was labelled with either “Donor 1” or “Donor 2”. Traceability of the blood donor is however maintained within a confidential clinical record, which may be accessed by the Chief Investigator, or by key members of the clinical team, to whom responsibility is delegated, if requested by the sponsor or other external bodies. After blood collection, treatment with artemether/lumefantrine (Riamet) was administered, followed by a two-week course of Primaquine. No supportive treatment or admission was required. Follow-up (clinic visits) until 90 days post-challenge was completed, with no safety concerns. Ongoing follow-up by email continued for 5 years with no evidence of relapsing malaria in either participant.

5.17. *P. vivax* PvW1

The blood donations from the *P. vivax*-infected participants in the VAC068 study were maintained at 37^{o}C and transported immediately to the Jenner Institute Laboratory, University of Oxford. All laboratory processing was conducted under Good Manufacturing Practice-like conditions, with Qualified Person and Quality Assurance (QA) oversight. Procedures were performed within fumigated microbiological safety cabinets, under precautions in compliance with Containment Level 3 Code of Practice and with sterile technique.

First, the red blood cells were separated from plasma by centrifugation of the leukodepleted blood before mixing with Glycerolyte 57 (at 1:2 erythrocyte to Glycerolyte 57 volume ratio). The first 20% of Glycerolyte 57 was added dropwise with gentle agitation, the suspension was then incubated for 5 minutes at room temperature before the remaining Glycerolyte 57 was added. The Red Blood Cell (RBC) Glycerolyte mixture was aliquoted into 1.5mL cryovials and frozen at -80^{o}C for one night before transfer to a dedicated liquid nitrogen tank. After 6 days, samples were transferred to Thermo Fisher Bishop’s Stortford temperature-monitored liquid nitrogen facility, Hertfordshire, UK, where samples were stored on behalf of the University of Oxford. In 2022 the inoculum was transferred back to University of Oxford in a continuous temperature monitored, controlled access cryostore. This process was performed under conditions similar

to those used for blood thawing in previous CHMI trials at Oxford and according to the Jenner Institute Laboratory and study specific Standard Operating Procedures (SOPs).

Confirmation of parasitic density within the blood collected for cryopreservation was performed via microscopy and qPCR on the leukodepleted blood samples. Thick film microscopy demonstrated 11 asexual parasites per 1µL leukodepleted blood for Donor 1, and 5 asexual parasites per 1µL leukodepleted blood for Donor 2. qPCR confirmed the presence of 23,566 genome copies/mL in Donor 1, and 14,078 genome copies/mL in Donor 2. This suggested minimal parasitic loss through the leukodepletion process when compared to the diagnostic qPCR values of 31,010 and 16,717 genome copies/mL, respectively.

Viability has also been demonstrated in an *ex-vivo* short-term culture of thawed infected red blood cells in enriched McCoy 5A medium. Parasitic growth was detectable by light microscopy, qPCR and flow cytometry through an initial 40-hour growth cycle in samples collected from Donor 1, with normal progression of normal morphology as seen on Giemsa stained thick and thin films. However, parasite growth was sub-microscopic in samples obtained from Donor 2. Therefore, it is the cryopreserved samples from Donor 1 that underwent further testing for sterility, mycoplasma, endotoxin and stringent safety (blood-borne infection) screening. Parasite DNA was then isolated and sequenced to produce a high-quality genome assembly. Importantly, no polymorphisms identified have been associated with resistance to sulfadoxine, chloroquine, artemether-lumefantrine or atovaquone-proguanil. This clone of *P. vivax* has been named PvW1.

The cryopreserved blood "inoculum" (from Donor 1) has since been used to infect healthy participants with *P. vivax* PvW1 in blood-stage CHMI studies (VAC069 Clinicaltrials.gov Identifier: NCT03797989, VAC071 Clinicaltrials.gov Identifier: NCT04009096, and VAC079 Clinicaltrials.gov Identifier: NCT04201431). These have included two Phase 1/2a clinical trials to assess vaccines targeting *P. vivax* Duffy-binding protein region II (PvDBPII).(53) Recombinant viral vaccines, using chimpanzee adenovirus 63 (ChAd63) and modified vaccinia virus Ankara (MVA) vectors, and a protein-in-adjuvant formulation (PvDBPII/Matrix-M) were tested in standard and delayed dosing regimens. Participants underwent blood-stage CHMI after their last vaccination, alongside unvaccinated controls. PvDBPII/Matrix-M, in a delayed dosing regimen, elicited the highest antibody response and reduced the mean parasite multiplication rate after CHMI by 51% (n = 6) compared with unvaccinated controls (n = 13) (Figure 4).(69)

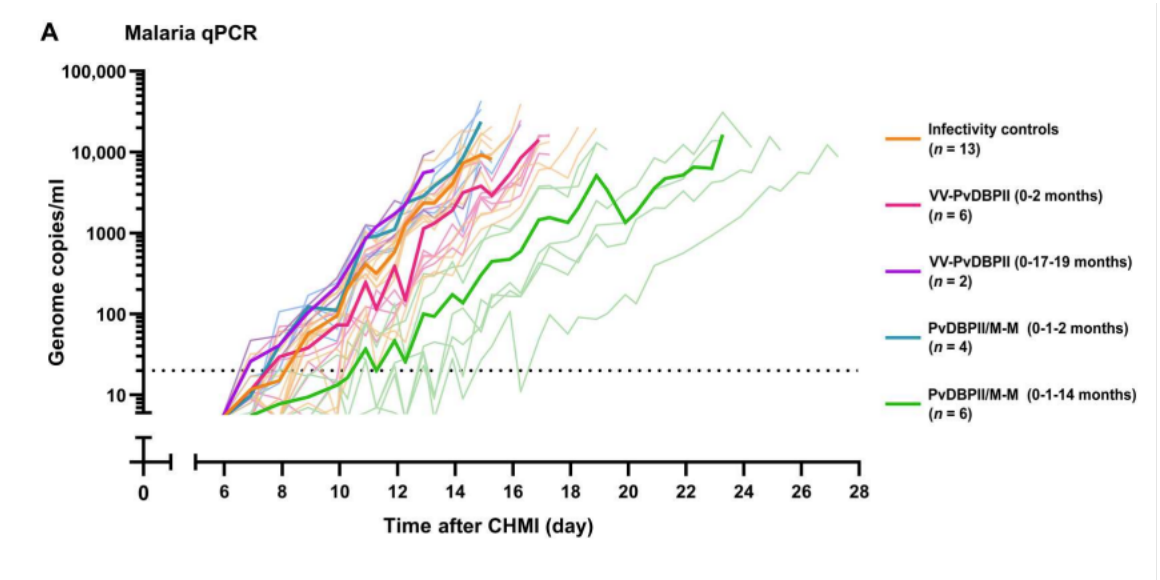


Figure 4: PvDBPII/Matrix-M inhibits growth of *P. vivax* after blood-stage CHMI. Individual parasitaemia over time was measured by qPCR, with group means in bold lines. Timings of vaccinations are shown in brackets in months. From Hou *et al.*(69)

5.18. Oxford experience of recurrent *P. vivax* infection

Oxford has also conducted a series of *P. vivax* re-challenge studies using the blood-stage inoculum, PvW1, to assess for development of immunity after successive exposure to a homologous *P. vivax* PvW1 infection (VAC069 Clinicaltrials.gov Identifier: NCT03797989). Here, malaria-naïve, Duffy blood group positive, healthy adults aged between 18 and 50 years were recruited to undergo CHMI by intravenous delivery of PvW1. The study was conducted in multiple phases, each corresponding to a CHMI. Participants who had undergone primary CHMI were invited to re-enrol and undergo secondary and tertiary homologous CHMI during subsequent phases of the study. In total, 19 participants underwent primary blood-stage *P. vivax* CHMI, 12 participants went on to complete secondary CHMI and 2 underwent tertiary CHMI. Results from the first four phases of the VAC069 study showed that repeat homologous CHMI with the same *P. vivax* clone did not induce anti-parasitic immunity (no significant effect on parasite growth upon repeat CHMI). However, following a single primary infection with *P. vivax*, clinical immunity was rapidly induced, as evidenced by reduced frequency and severity of clinical symptoms and laboratory abnormalities like lymphopenia during secondary CHMI compared to primary CHMI (Hou, Barber et al., manuscript in preparation).

Solicited adverse events were reported by the majority of participants following primary CHMI. About half of participants reported a grade 3 (severe) solicited adverse event during primary CHMI, which persisted at grade 3 for <48 hours. Fever (>37.5°C) occurred in the majority of participants following primary CHMI. Treatment associated solicited adverse events were uncommon and were of mild to moderate severity only. Unsolicited adverse events assessed as at least possibly related to CHMI were of maximal grade 2 severity with reduced appetite the most commonly reported symptom. Again, there were no SAEs related to CHMI.

The most common haematological laboratory abnormalities during primary CHMI were leucopenia, lymphopenia, anaemia and thrombocytopenia. Cell counts were lowest around the day of malaria diagnosis and recovered quickly except anaemia, which took longer to recover. There were two cases of grade 3 (severe) thrombocytopenia and five cases of grade 3 lymphopenia. These all returned to normal levels within a week without intervention. The most common biochemical laboratory abnormality was raised ALT. ALT peaked around day 6 after commencing anti-malarial treatment. Three participants developed raised ALT of grade 3 severity. They remained asymptomatic and had normal bilirubin and clotting results. ALT recovered to normal levels in all participants.

Both solicited and unsolicited adverse events, fever and laboratory abnormalities occurred less frequently and were less severe upon secondary CHMI compared to primary CHMI. Only two underwent tertiary CHMI limiting further comparisons.

5.19. Potential risks to participants

5.19.1 Phlebotomy

The maximum estimated volume of blood drawn over the study period should not compromise otherwise healthy participants. The actual total blood volume may vary depending on any requirement for repeat

clinical tests, e.g. in the event of a clinically significant abnormal result, the timing of malaria diagnosis and number of relapse infections.

Male regular blood donors may donate one unit (470mL) every 12 weeks and females every 18 weeks. However, a multicentre study by NHS Blood and Transplant (in Oxford and Cambridge) compared outcomes in 50,000 regular blood donors who were randomised to different intervals between blood donations (as regularly as giving 470mL every 8 weeks in male participant group and every 12 weeks in females) over 2 years.(70) There were no significant differences observed in quality of life, physical activity, or cognitive function although there were more symptoms related to blood donation, in groups donating more frequently, including tiredness, breathlessness, feeling faint, dizziness and restless legs. Symptoms were most increased among male participants. Lower mean haemoglobin and ferritin concentrations were also detected.

In this study, participants will never donate 470mL of blood in one sitting; the maximum volume they will donate in a single visit is 83mL so we would not expect them to report a high frequency of symptoms at the time of or just after blood donation. They will be closely monitored at all times of blood donation, and haemoglobin will be checked regularly, as described in the study procedures. Any abnormal result will be re-checked and referred to the GP for further investigation and management, as deemed clinically appropriate by the Investigator.

The approximate scheduled total blood volume drawn over the study period will be 477ml. An additional 35ml of blood may be drawn per relapse episode or assessment. Supplementary blood tests (such as repeat *P. vivax* qPCR to confirm negativity following treatment) may be performed at the discretion of the investigator. Although the additional effect of malaria infection on potential anaemia is acknowledged, we are confident that the anticipated total blood volume should not compromise our participants . Full blood count will be monitored throughout the challenge period. As an additional precaution, participants weighing less than 50kg at screening will be excluded from participation.

5.19.2 Venepuncture and cannulation

There may be minor bruising, local tenderness, pre-syncopal symptoms or syncope (rarely) associated with venepuncture or cannulation, which will not be documented as AEs if they occur. To reduce these risks, venepuncture and cannulation will be performed by appropriately trained staff members according to the local SOP.

5.19.3 Risk of other infections

The challenge agent in this study (i.e. the PvW1 isolate of *P. vivax* malaria) will be administered to healthy participants by mosquito bite. Although extremely unlikely, there is a theoretical risk of unintended transmission of other infectious agents.

The PvW1 isolate originated from a patient with *P. vivax* malaria in Thailand in 2018 who presented with fever to a southern field clinic in Songkhala. This initial source patient underwent testing for blood borne and mosquito borne infections. This included screening for antibodies to *Wuchereria bancrofti*, a nematode that is the major cause of lymphatic filariasis at the Thai field site. All results were negative.

The blood of this patient was fed to laboratory-reared mosquitoes, and these infected mosquitoes were then shipped from Thailand to the UK. A healthy UK participant was recruited as part of the VAC068 study (ClinicalTrials.gov Identifier: NCT03377296) (67) and underwent a malaria challenge administered by

mosquito bite. They were recruited and consented to act as a "donor" of a 250mL blood donation following the development of blood-stage *P. vivax* infection. Extensive screening for blood borne infections had also been performed and confirmed negative on this participant prior to mosquito-bite malaria challenge. For viral infections and syphilis, testing was either serological or by nucleic acid amplification methods, in accordance with the Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee guidelines.

After the UK participant had established blood-stage infection with PvW1, they donated 250mL of blood for creation of the PvW1 blood bank. This was created under strict GMP-like conditions as outlined in Section 5.17. It was subjected to direct screening and tested negative for bacterial contamination, endotoxin screen and mycoplasma specific culture. Plasma derived from the bank was also screened for blood borne infections and has tested negative for all blood-borne infection screens by PCR. Further to this, the blood donor also underwent repeat serological testing for HIV, Hepatitis B and C, syphilis, HTLV-1 and HTLV-2 90 days after malaria challenge. This was to ensure that no seroconversion from a recently acquired infection had occurred (that may have been undetectable around the time of challenge and therefore undetectable in the blood bank). All tests remained negative. Since the blood donor had been resident in Europe, where Bovine Spongiform Encephalopathy (BSE) has been reported, there is a theoretical risk of transmission of variant Creutzfeldt Jacob Disease (vCJD) from the inoculum. This risk was reduced by leukodepletion during the creation of the PvW1-infected blood bank. Leukodepletion is practised in UK blood donation, according to the Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee guidelines. Since universal leukodepletion was introduced in the UK in 1999, no cases of transmission of vCJD by transfusion have been recorded. (71) The PvW1 inoculum has since been administered intravenously to 37 healthy participants in Oxford without any safety concerns, specifically without any incidence of blood borne infection or vCJD.

As a precursor to this current study, the same PvW1 inoculum will be administered intravenously to healthy participants at Radboud University Medical Center (RUMC) in Nijmegen, Netherlands. These participants will undergo similar stringent safety testing. This will include screening for blood borne infections (HIV, Hepatitis B and Hepatitis C), West Nile virus (a mosquito borne disease found in Western Europe) by PCR test, and other relevant mosquito borne diseases if indicated by travel history. The blood of these participants will then be fed to mosquitoes, which will have been reared under carefully controlled laboratory conditions. It is these laboratory-reared mosquitoes which will bite the participants in our study in order to infect them with PvW1.

Due to this extensive repertoire of safety testing that has been performed on 1. the initial Thai source patient; 2. the UK human blood donor; 3. the PvW1 inoculum itself; and 4. the individuals undergoing blood-stage malaria challenge at RUMC to generate the infected mosquitoes, plus the fact that PvW1 will be administered by mosquito bite (rather than injection of human red blood cells), the risk of transmission of any infections other than PvW1 to our participants is extremely low.

5.19.4 Mosquito bites

Mosquito bites may cause local inflammatory reactions with redness, itching, swelling, scaling and/or tenderness. Topical anti-histamine cream for use twice daily for 3 days post-mosquito bite will be dispensed to both participants on the day of CHMI according to SOP OVG003: Medication Dispensing and Accountability. Serious allergic reactions including anaphylaxis have not been seen in CHMI studies to date,

but could theoretically occur. For this reason, participants will be inoculated in an area where Advanced Life Support trained physicians and defibrillator are immediately available.

5.19.5 *P. vivax* infection

Participants are likely to develop symptomatic malaria infection following CHMI, either at primary or relapse malaria infection. Anticipated symptoms, signs and laboratory findings may include feverishness, fever, tachycardia, hypotension, chills, rigors, sweats, headache, anorexia, nausea, vomiting, diarrhoea, myalgia, arthralgia, low back pain, thrombocytopenia and lymphopenia.(72) Unmonitored and untreated, *P. vivax* infection can be serious (rarely fatal) and, for this reason, participants will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the intensive follow-up schedule.

A very small proportion of participants in previous *P. vivax* blood-stage and sporozoite-stage CHMI studies have temporarily required intravenous fluid therapy for nausea and vomiting prior to treatment.(57, 73) If the Investigator judges the participant to be unwell enough to require continued intravenous fluids or more intensive medical input, they shall be transferred to the Infectious Diseases unit at the John Radcliffe Hospital under the care of the NHS Infectious Diseases team until they are well enough to be discharged home.

We expect participants to experience approximately 2-3 relapse infections during the 6-month relapse follow-up period (Figure 5).(31, 74) However, relapse infections may occur at any time during this period and the exact number to be expected is unknown. This uncertainty will be emphasised to participants. They will be advised to contact the study team if they develop any symptoms of relapse malaria infection so that prompt treatment may be initiated. Further information regarding safety considerations (including geographical restrictions) is outlined in section 10.2.

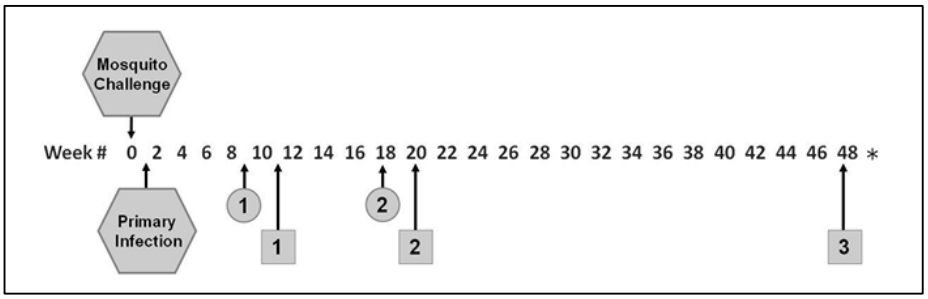


Figure 5: Timeline of relapsing infections in a previous *P. vivax* sporozoite CHMI study by Bennet *et al*.(31, 74) □ Subject A - poor metaboliser CYP2D6 phenotype; ○ Subject B - intermediate metaboliser CYP2D6 phenotype

5.19.6 Relapsing *P. vivax* malaria following Primaquine treatment

Infection with *P. vivax* malaria carries a risk of ongoing relapsing disease if anti-hypnozoite treatment is suboptimal. This risk is minimised by giving participants a 14-day course of high-dose Primaquine treatment (30mg once daily) at the end of in-person follow-up. This treatment will be observed as outlined in the study schedule to ensure compliance with therapy.

In a previous P. vivax sporozoite CHMI study, 2 participants (out of 33) experienced unexpected relapse infections following Primaquine treatment (Figure 5).(31, 74) These participants were found to have a CYP2D6 phenotype associated with either poor or intermediate metabolism of Primaquine into its active

metabolites. We will screen participants for their ability to metabolise Primaquine effectively by checking CYP2D6 genotype. Only participants who demonstrate “high-metaboliser” status will be enrolled into the study.

After completion of Primaquine treatment, participants will be followed up by email fortnightly until 1 year following CHMI and then annually thereafter until the End of Study (5 years following CHMI). These emails will inquire regarding any symptoms suggestive of relapse infection and any medical attendances. Participants will also be encouraged, for the duration of the study (i.e. 5 years), to contact the study investigators as soon as possible should they experience any symptoms in keeping with relapse malaria infection. If this occurs after the completion of Primaquine treatment, and it is logistically possible for the participant to attend the CCVTM, they will be reviewed by one of the study team, have a clinical assessment and an urgent blood sample taken for malaria qPCR. If this is positive and/or the participant is unwell and needs further clinical assessment the Chief Investigator will discuss with the Infectious Diseases consultant on call in Oxford University Hospitals (OUH) NHS Trust and arrangements will be made for them to be seen at the John Radcliffe hospital (and treated for relapse as soon as possible if a diagnosis has been confirmed). The DSMC will also be consulted in the event of any confirmed relapse following Primaquine treatment or clinical concern by the study team.

5.19.7 Incidental medical diagnoses

The medical tests carried out during the trial screening and follow-up have the potential to find incidental medical problems that may require referral of participants for further investigation. Participants will be informed of these, and, with their consent, their general practitioner will be contacted.

5.20. Potential benefits for participants

Participants will not benefit directly from participation in this study. However, it is hoped that their participation will contribute to the development of a safe and effective relapsing CHMI model which could contribute to the development effective *P. vivax* treatment and prevention therapies. Participants will also receive information about their general health status.

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint
Primary Objective To assess the safety, feasibility and frequency of relapsing *P. vivax* PvW1 infection after experimental sporozoite-administered Controlled Human Malaria Infection (CHMI)	Safety of primary and relapsing *P. vivax* infection following sporozoite-administered CHMI as measured by (S)AE occurrences	Throughout the study and by end of in-person follow-up period (month 7.5) Long-term safety data from remote follow-up period (month 8 – year 5)
	Primary *P. vivax* infection following sporozoite-administered CHMI as measured by detectable parasitaemia by qPCR +/- clinical symptoms	By day 21
	Frequency of *P. vivax* relapse infections as measured by number of malaria episodes confirmed by qPCR occurring within a 6-month follow-up period after treatment of primary infection, and time to relapse infection	By month 7
Secondary Objectives To assess the immune response to primary and relapsing *P. vivax* PvW1 infection	Serological response to a panel of *P. vivax* antigens by ELISA	C-2, C+7, C+14, C+21, at every fortnightly relapse clinic until C+196
Exploratory Objectives To assess the infectivity/transmissibility of primary and relapse *P. vivax* PvW1 infection following sporozoite-administered CHMI	A qPCR assay will be used to assess gametocyte induction following primary and each relapse *P. vivax* infection Cellular responses by flow cytometry	C-2, C+7, C+14, C+21, at every fortnightly relapse clinic until C+196

	Purified total IgG will be screened for functional anti-parasitic activity using growth inhibition assays.	

7. STUDY OVERVIEW

We will conduct an open-label experimental study to assess the safety and feasibility of introducing primary and relapsing *P. vivax* malaria infection following sporozoite CHMI administered by mosquito bite. Participants (N= up to 5) will undergo CHMI at the insectary facility of Radboud University Medical Center (RUMC) in Nijmegen, Netherlands. This will require a short trip (approximately 2 nights) to the Netherlands organised and accompanied by the University of Oxford study team. All other study visits and procedures will be conducted at the Oxford Vaccine Group (OVG), Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), University of Oxford. The trial is summarised in Table 2.

Participants will be telephoned daily for 6 days following CHMI before attending daily in-person visits for clinical review and blood sampling for *P. vivax* qPCR. Once primary *P. vivax* infection is confirmed, or a participant reaches day 21 following CHMI without detectable infection, participants will be treated with anti-malarial medication to clear primary blood-stage infection (Malarone or Riamet) but not Primaquine, which is necessary to clear the liver hypnozoites responsible for relapse *P. vivax* infections.

Participants will be required to attend a fortnightly "relapse clinic" where a blood sample will be tested by qPCR for *P. vivax* parasitaemia (Table 3). Participants will also be able to contact the study team at any time (i.e. 24/7) should they experience symptoms suggestive of a relapse malaria infection. If relapsing blood-stage *P. vivax* infection is confirmed, participants will be treated as before (with Malarone or Riamet but not Primaquine) and then re-commence fortnightly monitoring. At the end of the relapse follow-up period, all participants will be treated with Malarone (or Riamet) *and* Primaquine to clear any blood-stage infection and remaining hypnozoites respectively. Email follow-up will continue for 5 years.

Table 2: Study Overview

N=5	Month 0	Month 1 – Month 6.5	Month 6.5	Month 7.5	Month 8 – Month 12	Year 2 – Year 5
	In-person				Remote	
Study event	Sporozoite *P. vivax* CHMI	Relapse follow-up period	Definitive *P. vivax* treatment	Last in-person visit	Fortnightly email	Annual email
***P. vivax* infection**	Primary *P. vivax* infection	Relapse *P. vivax* infection(s)	-	-	-	-
Drug treatment	Malarone only	Malarone only	Malarone and Primaquine		-	-

Table 3: Study schedule

All participants (N=5)			
Timepoint	**Attendance**	**Timeline (days)**	**Visit**
Screening	1	-90	S
Pre-challenge	2	-2	C-2
CHMI	3	0	CHMI
	4 – 17	7 – 20	C+7 – C+20
Treatment of primary *P. vivax* infection (excluding Primaquine)	18	21	C+21/DoT[a]
	19	22	T+1
	20	24	T+3
	21	28	T+7
Relapse Clinic Follow-Up	22	28	C+28
	23	42	C+42
	24	56	C+56
	25	70	C+70
	26	84	C+84
	27	98	C+98
	28	112	C+112
	29	126	C+126
	30	140	C+140
	31	154	C+154
	32	168	C+168
	33	182	C+182
Anti-malarial treatment (including Primaquine)	34	196	C+196/DoT
	35	197	T+1
	36	199	T+3
	37	201	T+5
	38 – 42	203 – 212	T+7 – T+16
Last in-person visit	43	226	T+30
Relapse Assessment Visit	As required		RAx
Relapse Treatment Visits			RTx
			RTx+1
			RTx+3
			RTx+7

***S** = Screening; **CHMI** = Controlled Human Malaria Infection; **DoT** = Day of Treatment; **RAx** = Relapse Assessment visit; **RTx** = Relapse Treatment visit; **C-2** to **C+210** = Days in relation to day of CHMI; **T+1** to **T+30** = Days in relation to day of treatment; **RTx+1** to **RTx+7** = Days in relation to Relapse Treatment visit; **a** Day of treatment may occur earlier than C+21 per primary malaria infection treatment algorithm*

8. PARTICIPANT IDENTIFICATION

8.1. Study participants

We will recruit up to five healthy malaria-naïve UK adult participants (18-45 years) from Oxfordshire and the surrounding area.

We will recruit up to two additional "back up" participants. These participants will be on standby for the CHMI should a participant be unable to proceed with CHMI due to ineligibility, contraindication to CHMI or other reason including participant disinclination. Once the CHMI has been performed (point of enrolment) participants are unable to be replaced if they withdraw from the study. Back up participants will attend the C-2 visit and at least one of them will travel to RUMC, Nijmegen for the CHMI visit with the rest of the participants and the study team but will only undergo CHMI if required to replace a volunteer. If not required, their participation will end and they will receive compensation as outlined in Section 17.7.

We aim to enrol a mix of male and female participants. As *P. vivax* infection of reticulocytes is dependent on binding with the Duffy antigen, we will only enrol participants with a positive Duffy antigen phenotype. Participants with G6PD deficiency or a CYP2D6 genotype in keeping with poor or intermediate metabolism of Primaquine will not be enrolled. This is to ensure treatment with Primaquine is safe and effective respectively.

8.2. Inclusion criteria

The participant must satisfy all the following criteria to be eligible for the study:

- Healthy, malaria-naïve adult aged 18 to 45 years
- Able and willing to provide informed consent to participate in the study
- Able and willing (in the opinion of the Investigator) to comply with all study requirements
- Willing to allow the Investigators to access participant's electronic medical records or discuss the participant's s medical history with their GP
- Participants of childbearing potential only: must practice continuous highly effective contraception until 3 months after completion of Primaquine treatment (see Section 8.7)
- Negative haemoglobinopathy screen (including sickle cell disease and alpha and beta thalassaemia)
- Normal G6PD screen
- Agreement to refrain from blood donation until at least 3 years following completion of Primaquine treatment, as per current UK Blood Transfusion and Tissue Transplantation Services guidelines
- Able to answer all questions on the informed consent questionnaire correctly at first or second attempt
- Able to travel to CCVTM easily
- Able to travel to the Netherlands for malaria challenge with the necessary passport +/- visa requirements
- Reachable 24 hours a day by mobile phone during the period between CHMI and completion of Primaquine treatment

- Willing to take anti-malarial treatment for i) primary *P. vivax* infection ii) any relapse *P. vivax* infections and iii) at the end of relapse follow-up period as outlined in schedule of study procedures
- Willing to remain in Oxfordshire (or surrounding area) following malaria challenge (after return from the Netherlands) until completion of treatment of primary *P. vivax* infection
- Willing to remain within travelling distance of Oxfordshire (or surrounding area) following treatment of primary *P. vivax* infection until completion of Primaquine treatment (month 7). Absolute necessity is to remain on the UK mainland within 1-2 hours of secondary care NHS hospital. See Section 10.2
- Willing to be registered on the TOPS database (The Over volunteering Prevention System; www.tops.org.uk).

8.3. Exclusion criteria

The participant may not enter the study if ANY of the following apply:

- Red blood cells negative for the Duffy antigen/chemokine receptor (DARC)
- CYP2D6 genotype suggestive of poor or intermediate metabolism of Primaquine
- Body weight <50kg or Body Mass Index (BMI) <18.0 at screening
- History of clinical malaria (any species) or previous participation in any malaria vaccine trial or CHMI
- Travel to a clearly malaria endemic locality during the in-person study period or within the preceding six months
- Receipt of immunoglobulins or blood products (e.g. blood transfusion) in the last three months
- Receipt of an investigational product in the 30 days preceding enrolment (or planned receipt during the study period) likely to impact on interpretation of the trial data or the *P. vivax* parasite as assessed by the Investigator
- Concurrent involvement in another clinical trial involving an investigational product or planned involvement during the study period
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)
- Any history of severe allergy or anaphylaxis
- Use of systemic antibiotics with known anti-malarial activity within 30 days of CHMI (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin)
- Use of anti-malarials within 30 days of CHMI
- Any clinical condition known to prolong the QT interval
- History of cardiac arrhythmia, including clinically relevant bradycardia
- Disturbances of electrolyte balance, e.g. hypokalaemia or hypomagnesaemia
- Family history of congenital QT prolongation or sudden death
- An estimated ten-year risk of fatal cardiovascular disease of ≥5% at screening, as determined by the Systematic Coronary Risk Evaluation (SCORE2) shown in Appendix A

- Use of medications known to have a potentially clinically significant interaction with both Riamet and Malarone
- Use of medications known to have a potentially clinically significant interaction with Primaquine
- Any other contraindications/known hypersensitivities to both Riamet and Malarone
- Any other contraindications/known hypersensitivities to Primaquine
- History of sickle cell anaemia, sickle cell trait, thalassaemia or thalassaemia trait, G6PD deficiency or any haematological condition that could affect susceptibility to malaria infection
- Pregnancy, lactation or intention to become pregnant during the study
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)
- History of serious psychiatric condition that may affect participation in the study
- Any other serious chronic illness requiring hospital specialist supervision
- Suspected or known current alcohol misuse
- Suspected or known injecting drug use in the 5 years preceding enrolment
- Hepatitis B surface antigen (HBsAg) detected in serum
- Seropositive for hepatitis C virus (antibodies to HCV) at screening (unless participant has taken part in a prior hepatitis C vaccine study with confirmed negative HCV antibodies prior to participation in that study, and negative HCV ribonucleic acid (RNA) qPCR at screening for this study)
- Participants unable to be closely followed for social, geographic or psychological reasons.
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, or clinical examination. The normal range of results for each blood parameter is shown Appendix B. In the event of abnormal test results, confirmatory repeat tests will be requested. Procedures for identifying laboratory values meeting exclusion criteria are described in Appendix B.
- Any other significant disease, disorder, or finding (in the opinion of the Investigator) which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data
- Inability of the study team to confirm medical history via electronic records or contact the participant's GP to confirm medical history

8.4. Contraindications to CHMI

The following constitute contraindications to CHMI:

- Acute disease, defined as moderate or severe illness with or without fever
- Pregnancy
- Any other significant disease, disorder, or finding (in the opinion of the Investigator) which may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to participate in the study or impair interpretation of the study data

8.5. Other concomitant medication

Participants will not be enrolled if they are taking medications known to have a potentially clinically significant interaction with both Malarone and Riamet, or Primaquine. If a participant requires treatment with a medication which is known to interact with Malarone, they will be treated with Riamet as an

alternative anti-malarial medication. A decision to withdraw the participant will be at the discretion of the Investigators.

8.6. Prevention of over-volunteering

Participants will be excluded from the study if they are concurrently involved in another trial involving an investigational product. Participants will be asked to provide their National Insurance or Passport number (if they do not have a National Insurance number) and they will be registered on a national database of participants in clinical trials (www.tops.org.uk) to prevent over-volunteering.

8.7. Contraception

Participants who are of childbearing potential will be required to use a highly effective form of contraception as *P. vivax* infection (primary or relapse) could pose a serious risk to both maternal health and the unborn fetus. In addition, Primaquine treatment is contraindicated in pregnancy.

A participant of childbearing potential is defined as any participant who has experienced menarche and who is NOT:

- Surgically sterile (including hysterectomy, bilateral tubal ligation, or bilateral oophorectomy)
- OR post-menopausal (defined as amenorrhea for at least 12 consecutive months without an alternative medical cause)

The requirement for highly effective contraception will be from date of screening until 3 months after completion of Primaquine treatment (i.e. Approximately 10 months following malaria challenge).

Highly effective forms of contraception include:

- Established use of oral, intravaginal or transdermal combined hormonal methods of contraception
- Established use of oral, injected or implanted progesterone-only hormonal contraception associated with inhibition of ovulation e.g. oral desogestrel, injectable depot medroxyprogesterone acetate, or subdermal implantable etonogestrel
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- Bilateral tubal occlusion or total hysterectomy.
- Male sterilisation, if the vasectomised partner is the sole partner for the subject.
- True abstinence (as defined as refraining from heterosexual intercourse), when this is in line with the preferred and usual lifestyle of the subject (periodic abstinence and withdrawal are not acceptable methods of contraception).

Barrier methods of contraception (e.g. condom or occlusive cap with spermicide) may be used in combination with any of the above forms of contraceptive but are not considered highly effective methods if used as the sole method of contraception. If Riamet (artemether/lumefantrine) is used as the anti-malarial treatment for primary or relapse malaria infection, participants using hormonal contraceptives must agree to using an additional method of contraception (e.g. barrier methods) from the time of commencing the Riamet drug course until the next menstrual period. This is to minimise any risk of possible reduced effectiveness of hormonal contraceptives during concomitant anti-malarial therapy, due to the weak induction of cytochrome P450 enzymes resulting from treatment with artemether.

The procedure to be followed if a participant becomes pregnant during the study period is outlined in Section 10.5.

9. PROTOCOL PROCEDURES

9.1. Schedule of study procedures

An overview of study procedures is outlined below (Tables 4 – 7).

Table 4: Schedule of events for screening

	Screening
Attendance number	1
Timeline (days)	S
Window	0 - 90 days before CHMI
Informed consent	X
Informed consent questionnaire	X
Demographic details recorded	X
Inclusion/Exclusion criteria	X
Medical history/examination	X
Review medications	X
Physical observations*	X
TOPS eligibility check and registration	X
Emergency contact details recorded	X
Electrocardiogram	X
Urine β-hCG**	X
Haematology (mL)#	2
Biochemistry (mL)##	5
HIV, Hepatitis B, Hepatitis C, EBV, CMV serology (mL)	5
Haemoglobinopathy screen (mL)	2
G6PD screen (mL)	2
Duffy antigen phenotyping (mL)	2
CYP2D6 metabolism screen (mL)	2
Total Blood Volume (mL)^	**20**
Cumulative blood volume (mL)^	**20**

***S** = Screening; **CHMI** = Controlled Human Malaria Infection; ***Physical observations will include termperature, heart rate, blood pressure, height and weight. ****For participants of childbearing potential only. **#** Haematology will comprise full blood count. **##** Biochemistry will include sodium, potassium, urea, creatinine, magnesium, liver function tests, albumin and serum cholesterol. **^** Blood volumes as given in the Table are approximate volumes*

Table 5: Schedule of events for *P. vivax* CHMI

	Pre-CHMI	CHMI		Treatment							
Attendance number	2	3	-	4	5-10	11	12-17	18	19	20	21
Timeline (days)	C-2	CHMI	C+1-C+6	C+7	C+8-C+13	C+14	C+15-C+20	C+21/DoT[a]	T+1	T+3	T+7
Window	-1	0	0	0	0	0	0	0	0	0	±1
Telephone call			X								
Inclusion/Exclusion criteria	X	X									
Medical history/examination	(X)	(X)		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Review medications	X	X		X	X	X	X	X	X	X	X
Physical observations*	X	X		X	X	X	X	X	X	X	X
Collect solicited AEs				X	X	X	X	X	X	X	X
Collect unsolicited AEs		(X)		X	X	X	X	X	X	X	X
Collect MAAEs and SAEs	(X)	(X)		X	X	X	X	X	X	X	X
Review contraindications	X	X						X			
Sporozoite CHMI		X									
Medic alert card provided		X									
Malarone or Riamet issued								X	X		
Urine β-hCG[**]								X			
Immunology (mL)	64			10		10		60			
qPCR & gametocyte PCR (mL)	2			2	2	2	2	2		2	2[f]
Tempus tube (RNA)	3									3	
Haematology (mL)[#]	2			2		2		2		2	2
Biochemistry (mL)[##]	5			5		5		5		5	5
Serum βhCG (mL)[**]	5			5		5		5			
Total Blood Volume (mL)^	**81**	**0**	**0**	**24**	**12**	**24**	**12**	**74**	**0**	**12**	**9**
Cumulative blood volume (mL)^	**101**	**101**	**101**	**125**	**137**	**161**	**173**	**247**	**247**	**259**	**268**

Table 6: Schedule of events for relapse follow-up period

	Relapse clinic													Relapse Assessment +/- Treatment					
Attendance number	22	23	24	25	26	27	28	29	30	31	32	33		As required[y]					
Timeline (days)	C+28	C+42	C+56	C+70	C+84	C+98	C+112	C+126	C+140	C+154	C+168	C+182		Pre-RAx	RAx	RTx	RTx+1	RTx+3	RTx+7
Window	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2		0	0	0	0	0	±1
Telephone call														(X)					
Medical history/examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)		(X)	X	(X)	(X)	(X)	(X)
Review medications	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X		X[e]	X	X	X	X	X
Collect solicited AEs	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Collect unsolicited AEs														X	X	X	X	X	X
Collect MAAEs and SAEs	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Review contraindications																X			
Malarone or Riamet issued																X	X		
Urine β-hCG**	X	X	X	X	X	X	X	X	X	X	X	X				X			
Immunology (mL)	4	4	4	4	4	4	4	4	4	4	4	4							
qPCR & gametocyte PCR (mL)	2	2	2	2	2	2	2	2	2	2	2	2			2			2	2[f]
Tempus tube (RNA)	3	3	3	3	3	3	3	3	3	3	3	3						3	
Haematology (mL)[#]															2			2	2
Biochemistry (mL)[##]															5			5	5
Serum βhCG (mL)**															5				
Total Blood Volume (mL)^	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**		**0**	**14**	**0**	**0**	**12**	**9**
Cumulative Blood Volume (mL)^	**277**	**286**	**295**	**304**	**313**	**322**	**331**	**340**	**349**	**358**	**367**	**376**		**0**	**14**	**14**	**14**	**26**	**35[y]**

Table 7: Schedule of events after relapse follow-up period

	Treatment		Primaquine						Last in-person visit	Fortnightly email	Annual email
Attendance number	34	35	36	-	37	-	38	39-42	43	-	-
Timeline (days)	C+196/DoT	T+1	T+3	T+4	T+5	T+6	T+7	T+8 - T+16[c]	T+30/C+226	C+240 - C+366	C+2 - C+5 years[d]
Window	±2	0	0	±1	±1	±1	±1	±1	+14	±3	±28
Telephone call				X		X		(X)	(X)	(X)	(X)
Email										X	X
Medical history/examination	(X)	(X)	(X)		(X)		(X)	(X)	(X)	(X)	(X)
Review medications	X	X	X	X	X	X	X	X	X	X	X
Physical observations*	X	X	X		X		X	X[e]	X[e]		
Collect solicited AEs	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X	X
Collect unsolicited AEs	x	x	x	x	x	x	x	x	X		
Collect MAAEs and SAEs	X	X	X	X	X	X	X	X	X	X	X
Review contraindications	X		X								
Malarone or Riamet issued	X	X									
Primaquine issued			X		X		X	(X)			
Urine β-hCG**	X										
Immunology (mL)	64										
qPCR & gametocyte PCR (mL)	2		2				2	(2)[f]			
Haematology (mL)#	2		2				2				
Biochemistry (mL)##	5		5				5				
HIV, Hepatitis B, Hepatitis C, EBV, CMV serology (mL)	5										
Serum βhCG (mL)**	5										
Total Blood Volume (mL)^	**83**	**0**	**9**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**
Cumulative Blood Volume (mL)^	**459**	**459**	**468**	**468**	**468**	**468**	**477**	**477**	**477**	**477**	**477**

S** = Screening; **CHMI** = Controlled Human Malaria Infection; **DoT** = Day of Treatment; **Pre-RAx** = Pre-Relapse Assessment visit; **RAx** = Relapse Assessment visit; **RTx** = Relapse Treatment visit; **C-2** to **C+366** = Days in relation to day of CHMI; **T+1** to **T+30** = Days in relation to day of treatment; **RTx+1** to **RTx+7** = Days in relation to Relapse Treatment visit; **(X)** = If considered necessary, emphasising any acute complaints. ** Physical observations will include termperature, heart rate and blood pressure. ****** For participant of childbearing potential only. **#** Haematology will comprise full blood count. **##** Biochemistry will include sodium, potassium, urea, creatinine, liver function tests and albumin. **^** Blood volumes as given in the Tables are approximate volumes*

***a** Day of treatment may occur earlier than C+21 per primary malaria infection treatment algorithm. **c** Participants wil be followed up daily for the duration of Primaquine treatment (until T+16). They will be seen at least 3 times a week in person for direct observation of treatment. On days when they are not attending clinic, they will be telephoned to confirm they have taken the dose of Primaquine. **d** Annual email follow-up from date of CHMI. **e** Physical observations will be performed at in-person visits only. **f** Blood sampling for qPCR may continue up to 48 hourly until the participat has at least one negative qPCR reading (<20 genome copies/mL) at the discretion of the Investigator**. y** Participants will undergo a relapse assessment if they have i) symptoms suggestive of relapse malaria infection or ii) parasitaemia detected on qPCR from relapse clinic blood samples. See relapse assessment pathway and treatment algorithm. Participants with relapse malaria infection will receive anti-malarial treatment and attend relapse treatment visits (RTx, RTx+1, RTx+3, RTx+7) in place of relapse clinic visits. After RTx+7, participants will attend the next relapse clinic appointment which is due based on their current timepoint following CHMI (C+ days) and continue fortnightly relapse clinic follow-up thereafter.*

9.2. Recruitment

Advertisements for recruitment will be distributed through methods including but not limited to posters, leaflets, websites, newspapers, radio, public engagement events, and/or social media, using advertising material containing wording from approved study documents to invite participation in the study. Potential participants may be contacted by methods including but not limited to email, telephone, and/or mail, using an approved invitation letter.

Where mail-outs are used, participants may be identified via the electoral open register or they will be contacted via their GP surgeries.

For mail-outs via the electoral register, the study team will obtain access to the names and addresses of individuals who are on the open electoral register (which contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH Docmail system (or equivalent company), and the study invitation pack will be sent out by CFH Docmail (or equivalent company).

The details of other recruitment methods which may be used are outlined below:

- **Email campaign**: We may contact representatives of local tertiary education establishments and local employers and ask them to circulate approved posters and a link to the study website by email or hard copy.
- **Volunteers' databases**: Direct email and link may be sent to members of the public who have registered their interest in potentially volunteering for clinical trials. These are secure databases where members of the public registered here have given consent to have their details recorded and be contacted expressly for the purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to participating for any trial they are contacted about. Further information on Be Part of Research Volunteer Service (as one of these methods) can be found in appendix D
- **Media advertising**: Approved local media, radio, streaming services (such as Spotify), newspaper and website advertisements may be placed/announced in locations relevant for the target age group with brief details of the study and contact details for further information.
- **Website advertising**: Description of the study and copy of the information booklet may be placed on study websites and other appropriate platforms for vaccine trial advertising. Individuals can indicate their interest and could be followed up to answer any questions about the study without committing to participate in any studies (warm calling). Google advertisements including banner advertisements on third party websites can be used.
- **Social media**: Approved advertisements may be placed on trial social media accounts or targeted social media platform advertisements including, but not restricted to Twitter/X, Facebook and Instagram. Posts on social media accounts owned and operated by the Investigators and related study team(s).
- **Exhibitions/Presentations**: Advertising material and/or persons providing information relating to the study may exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs. Investigators or members of study team or research ambassadors (previous study participants who have agreed to be contacted about future studies) may provide information

about the study via presentations (e.g. presentations at lectures, invited seminars, online webinars) or videos.

- **Poster/Leaflet in public places:** Advertising material may be displayed in public places, including public transport, community centres/noticeboards and other venues, with the agreement of the owner/proprietor.
- **SMS/text messages**: SMS/text message (or emails) may be sent to potential participants identified by GPs from their databases (which will require Participant Identification Centres [PIC] agreements to be set up with the GP surgeries).
- **Royal Mail Leaflet**: Royal Mail door-to-door service with delivery of invitation letters enclosed in envelopes may be sent to every household within certain postcode areas.

9.3. Online Screening Questionnaire

Information about the study will direct volunteers to the study website, where a full participant information sheet will be available. The website will also contain contact details of the study team should potential participants wish to ask any questions. Volunteers who are interested in participating will be asked to complete an initial online questionnaire which will include initial eligibility screening and e-consent for permission i) to store personal information, ii) to be contacted for the purposes of this clinical trial and iii) to obtain and store relevant personal medical information (either by accessing electronic health records or contacting their GP practice) for the purpose of eligibility assessment, before they are invited for a full screening and consent visit.

If a potential participant is not able or willing to complete the online screening questionnaire (but is willing to proceed with an in-person visit) they can be invited to attend a face-to-face screening.

9.4. Informed Consent

The Participant Information Sheet (PIS) and Informed Consent Form (ICF) will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part in the study. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal. The PIS will be made available to the volunteer at least 24 hours prior to the screening visit. Videos, audio, and animations may be used to make the Participant Information Sheet and Informed Consent Form more accessible.

At the screening visit, the volunteer will be fully informed of all aspects of the study including the aims of the study, details of any tests being performed, the potential risks and the implications and constraints of participation. The following will be emphasised:

- Participation in the study is entirely voluntary.
- Refusal to participate involves no penalty or loss of medical benefits.
- Participants may withdraw from the study at any time.
- They are free to ask questions at any time to allow them to understand the purpose of the study and the procedures involved.
- There is no direct benefit from participating.

- Their general practitioner (GP) or electronic health records will be consulted to corroborate their medical history and confirm that they are eligible to take part in the study.
- Participants will be registered on the TOPS database (The Over-volunteering Prevention System; www.tops.org.uk).
- Blood samples will be collected, and, with their consent, any leftover samples may be stored indefinitely for use in other, ethically approved research.

The participant will be allowed as much time as necessary to consider the information. They will be allowed to question the Investigator, their GP, or other independent parties to decide whether or not they wish to participate in the trial. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtains consent must be suitably qualified and experienced, and authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site. The participant must personally sign and date the latest approved version of the Study ICF before any study specific procedures are performed.

9.5. Informed Consent Questionnaire

Prior to completion of the Informed Consent Form (ICF), Participants attending the screening visit will be asked to complete an Informed Consent Questionnaire (ICQ) testing their understanding of the study. This helps to ensure that individuals understand the study sufficiently to give informed consent. If the participant successfully answers all the questions correctly within the ICQ, and decides to participate in the study, they will then proceed to sign the ICF. Participants who fail to answer all questions correctly on their first attempt will be allowed to re-take the questionnaire one more time, following further discussion with the Investigator. Provided they subsequently answer all questions in the ICQ correctly on a second attempt they may then complete the ICF.

9.6. Screening and Eligibility Assessment

Once written informed consent has been received, the following baseline assessments will be performed as outlined in schedule of procedures Table 4 (Section 9.1) will be performed and recorded as part of the assessment of inclusion/exclusion criteria:

- Participant demographics: e.g., age, sex, and ethnicity
- Medical history
- Contraception: participants of childbearing potential will be asked if they are willing to use effective contraceptive measures as outlined in Section 8.7
- Use of concomitant medication and vaccinations (including over the counter medications, vitamins, recreational drug use and herbal supplements)
- Recording of resting pulse rate, blood pressure, temperature, weight, and height
- Physical examination including (but not limited to) cardiovascular, respiratory and abdominal examination.
- Urine pregnancy test (participants of childbearing potential only)
- Blood samples: full blood count; urea and electrolytes; liver function tests; albumin; serology for HIV, hepatitis C and hepatitis B, EBV and CMV; Duffy antigen phenotype; CYP2D6 Primaquine metabolism screen

- Electrocardiogram
- Next of kin contact details

Medical history, including vaccination and prescribed medication, will be collected by participant recall and verified from GP records or electronic healthcare records (if available). The participant's GP will be notified of an individual's participation in the study.

Some individuals may test positive for hepatitis C virus antibodies due to previous involvement in a hepatitis C vaccine study. If this applies to an individual, with the consent of the participant, the team responsible for the hepatitis C vaccine study will be contacted to verify the participant's hepatitis C status prior to enrolment in this study. Additional tests (e.g. hepatitis C qPCR) may be performed, with the consent of the participant, at the discretion of the Investigator.

The maximum length of time permitted between screening and CHMI is 90 days. Where more than 90 days has lapsed, a re-screening visit will be conducted to ascertain if there have been any changes in the medical history or physical examination findings. Biochemistry and haematology blood tests will be repeated. Other blood tests and electrocardiogram will not be repeated routinely. The GP (or electronic) health records will not necessarily need to be re-checked unless there is any need to obtain further updated information (e.g. regarding a new medical event or finding). This will be at the discretion of the Investigator.

To avoid unnecessary additional procedures, if the appropriate screening information (including investigation results and GP summary) are available for a participant from a screening visit of another study, these results may be used to assess eligibility, provided screening assessment occurred within 90 days of CHMI.

9.7. Group Allocation

There are no groups in this study. All participants will undergo the same study procedures as outlined in Section 9.1.

9.8. Blinding and code-breaking

This is an unblinded study. There are no blinding or code-breaking procedures to describe.

9.9. Study interventions

9.9.1 Sporozoite *P.vivax* PvW1 CHMI

The infected *Anopheles stephensi* mosquitoes for the sporozoite CHMI will be produced in the RaViCHMI study being conducted by RUMC.

In brief, the RaViCHMI study involves a blood-stage CHMI using *P. vivax* PvW1 inoculum (Section 5.17) administered to healthy adult participants by intravenous injection. Once the participants develop detectable malaria infection (and prior to curative anti-malarial treatment), batches of laboratory-reared *Anopheles stephensi* mosquitoes will feed on the participant blood in order to infect the mosquitoes with PvW1. This will be conducted using an optimised combination of gametocyte-sparing treatment, ex vivo

gametocyte concentration techniques, and blood-feeding techniques (including direct skin feeding and membrane feeding) to maximise the infection rate of the mosquitoes.

The blood-fed mosquitoes will be stored in appropriate conditions to allow the development of *P. vivax* sporozoites in the salivary glands of the mosquitoes. Samples from the batches of mosquitoes will undergo dissection to confirm infection. All these procedures will be conducted in accordance with local SOPs and the RaViCHMI study protocol at RUMC.

For the sporozoite CHMI in this study, all participants recruited in Oxford, UK (N=5), plus "back-up" participant(s), will attend the RUMC insectary on a designated day. The CHMI is considered point of enrolment. The eligibility of participants to proceed with the CHMI will be reviewed 2 days prior to CHMI (in Oxford) and on the day of CHMI (at RUMC). If a participant is not eligible to proceed or has a contraindication to CHMI (Section 8.4) on the day of CHMI, a "back up" participant will instead undergo CHMI. An ineligible participant will not proceed any further in the study.

On the day of CHMI, participants will be briefed regarding local safety rules. An appropriately trained researcher will prepare the mosquitoes for sporozoite CHMI per local SOPs. Small containers of mosquitoes will be covered with netting and held in a second container to minimise any possibility of escape.

The sporozoite CHMI will be administered by exposure of participants to five infectious mosquito bites. Initially, participants will be exposed to the bites of five infectious mosquitoes by placing their forearms over the container of mosquitoes for 5-10 minutes. Fed mosquitoes (as indicated by the presence of a blood meal in the abdomen) will be individually dissected immediately by the trained RUMC researchers. They will then be assessed for sporozoite load in the salivary gland (graded 0 to +4). A gland rating of +2 or more, representing 10 or more observed sporozoites, qualifies as being "infectious". If, by this method the participant is found to have been inoculated by fewer than five infected mosquitoes, further mosquitoes are allowed to feed on the participant until a total of 5 appropriately infected mosquitoes have fed. The bite-challenge procedure continues until the subject has been bitten by 5 infectious mosquitoes.

The rationale for 5 infectious mosquito bites is based on the extensive previous experience with *P. falciparum*, where sporozoites inoculated by < 5 mosquitoes have led to an irregular infection in malaria-naive human participants. However, this does not appear to be the case for *P. vivax*: 100% of the 33 participants (controls and vaccinees) bitten by five *P. vivax*-infected mosquitoes in the WRAIR US study developed patent parasitaemia, and also of the 53 non-vaccinated participants (either malaria-naïve or semi-immune) bitten by between 2 and 4 infected mosquitoes in the Cali studies, 52 developed patent parasitaemia (about 100-1000 sporozoites are inoculated per bite). However, to ensure reliable infection in our small study of just 5 participants we have chosen to proceed with 5 infectious bites.

The challenge will be performed in an area of the insectary that is isolated by appropriate and approved mechanisms according to local SOPs. Equipment and procedures to manage an unexpected medical emergency (e.g. adrenaline for anaphylaxis) will be in place in addition to suitably qualified medical professionals.

9.10. Study Visits

The procedures performed at each study visit are documented in the schedule of study procedures (Section 9.1). Each visit is assigned a time-point and a window period within which the visit may be

conducted. Whether a visit can occur out of window will be decided on a case-by-case basis by the study investigators.

The CHMI will take place at Radboud University Medical Center (RUMC) in Nijmegen, Netherlands. All other study visits (including screening and C-2 visits) will be conducted at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) at the University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE.

9.11. Pre-CHMI Review (C-2)

This visit will include a reassessment of eligibility to undergo CHMI by review of study inclusion and exclusion criteria and review of any contraindications to CHMI (Section 8.4). Any new medical issues or symptoms that have arisen will be assessed by medical history and examination (if required). Physical observations, safety bloods, serum β-hCG in participants of childbearing potential and malaria qPCR will be undertaken according to Table 5.

The C-2 visit will also include:

- Documentation of full contact details for each participant including:
  - mobile number (verified by test phone call)
  - landline number (if applicable)
  - work telephone number (if applicable)
  - home address (and address of any temporary residence if applicable)
  - work address (if applicable)
- Documentation of name and 24-hour telephone number of a close friend, relative or housemate who will be kept informed of their whereabouts for the duration of the study
- Documentation of name and 24-hour telephone number of next of kin to be contacted in case of emergency
- Review of concurrent medication including over the counter medication

9.12. CHMI

The CHMI will be conducted on the same day for all participants at Radboud University Medical Center (RUMC) in Nijmegen, Netherlands. For details of the administration of sporozoite *P. vivax* CHMI see Section 9.9.1.

Travel and accommodation will be organised by the study team and clear instructions will be provided to the participants. Participants will be required to fulfil the necessary passport and/or visa requirements to travel to the Netherlands. They will be advised to arrange a UK Global Health Insurance Card (GHIC) or European Health Insurance Card (EHIC) and personal travel insurance which can be reimbursed by the study team. In the event of cancellation or unexpected extension of stay, travel and accommodation costs will be organised by the study team.

It is anticipated that travel to Nijmegen will occur the day prior to malaria challenge, while return travel to Oxford will take place the day following malaria challenge (i.e. two nights of accommodation in Nijmegen). However, the exact travel arrangements may be subject to availability. Members of the study team will accompany the participants and be responsible for their safety and welfare throughout this time, ensuring

safe return to Oxford. Participants will be informed that it will not be possible to extend their travel or participate in activities beyond the scope of the malaria challenge.

The CHMI visit will include:

- A confirmation of eligibility to undergo CHMI by review of study inclusion and exclusion criteria and review of any contraindications to CHMI (Section 8.2 - 8.4)
- Assessment of any new medical issues or symptoms by medical history and examination (if required)
- Review of concurrent medication including over the counter medication
- Baseline physical observations (pulse, blood pressure and temperature)
- Sporozoite *P. vivax* CHMI (Section 9.9.1)
- Provision of a Medic-Alert type card to all participants. This will contain the contact details of the study physician and a reminder that the research team be contacted immediately in the event of illness, accident or symptoms of relapse *P. vivax* infection
- Provision of an accurate oral thermometer

Participants must be resident in Oxfordshire or the surrounding area following return from the Netherlands until completion of primary *P. vivax* anti-malarial treatment. Participants will be counselled that should they fail to return for treatment after the challenge, they could become very unwell and potentially die. They will be informed that should they fail to attend a scheduled clinic visit following CHMI, and be uncontactable by telephone, their nominated contact and/or next of kin will be contacted. If their whereabouts cannot be determined, the police and media may be informed in order to start a search and ensure their safety.

9.13. CHMI Days 1 – 6: Daily Telephone Reviews

Parasitaemia (detectable by qPCR) is not expected until at least 7 days after sporozoite *P. vivax* CHMI. Therefore, participants will not need to be reviewed in clinic until day 7 (C+7) after CHMI. They will be telephoned daily by the clinic team from day 1 to 6 to make sure they are well and contactable. This may be performed in person (e.g. on day 1 following CHMI). Participants will also be able to contact the study physicians on the 24-hour emergency telephone number if needed.

9.14. CHMI Days 7 – C+21/Day of Treatment: Daily Clinic Reviews

Participants will be reviewed in clinic once daily from day 7 until day 20 or until diagnostic criteria are met. Each follow-up visit will include:

- Assessment of any new medical issues or symptoms by medical history and examination (if required)
- Review of concurrent medication including over the counter medication
- Physical observations including temperature, pulse rate and blood pressure
- Collection of solicited Adverse Events (AEs) i.e. questioned if they have experienced any foreseeable symptoms of malaria (Section 11.2)
- Collection of unsolicited AEs
- Collection of Medically-Attended AEs (MAAEs)
- Collection of any Serious Adverse Events (SAEs)

- Blood tests (including daily qPCR to assess for *P. vivax* parasitaemia) per the study schedule of events (Table 5)

Participants will be encouraged to contact one of the Investigators on the 24-hour study mobile telephone if they develop symptoms of malaria or concerning AEs between the regular clinic reviews. The severity of symptoms will be assessed using grading criteria summarised in Section 11.

Primary *P. vivax* malaria infection will be diagnosed according to criteria described in Section 9.15.

If a participant is diagnosed with primary *P. vivax* infection prior to visit C+21, they will no longer need to be seen at the daily clinics and instead move on to the treatment visit schedule (i.e. DoT, T+1, T+3…). If a participant reaches day 21 following CHMI (C+21) without meeting diagnostic criteria, they shall be started on anti-malarial treatment empirically on this day. They will continue the study schedule as normal after this.

If a participant is unwell and unable to attend CCVTM for a clinic visit, they will be reviewed by telephone by a study clinician. If in-person assessment is considered necessary, the participant will be referred to appropriate NHS services. If there are any severe complaints not typical for malaria infection, such as chest pain, the participant will be evaluated by a qualified clinician using the appropriate clinical assessments (e.g. ECG or measurement of cardiac enzymes) according to standard hospital care. Discussion with the Chief Investigator and any necessary referrals to secondary care will be made accordingly.

9.15. Primary *P. vivax* infection – Diagnosis and treatment Algorithm

Real-Time quantitative PCR (qPCR) will be used as the diagnostic tool for assessment of primary and relapsing *P. vivax* infections. This is described in more detail in Section 9.24.2.

A positive diagnosis of primary *P. vivax* infection following CHMI (C+7 – C+20) will be made once a participant has detectable parasitaemia >1000 genome copies per ml regardless of the presence or absence of malaria symptoms (Figure 6) (Section 11.2). Following diagnosis, the participant will be initiated on anti-malarial treatment (Section 9.16) to treat blood-stage *P. vivax* infection. This will usually commence the following morning (Day of Treatment (DoT)) unless there is clinical concern prior to the qPCR result being available.

If a participant is diagnosed with primary *P. vivax* infection prior to visit C+21, they will no longer need to be seen at the daily clinics and instead move on to the treatment visit schedule (i.e. DoT, T+1, T+3…).

Investigators can treat any participant for malaria at any time following CHMI (regardless of the qPCR result) if they have clinical concerns and have discussed the case with the Chief Investigator. If necessary, participants can be discussed with DSMC or the Infectious Diseases Consultant on call at the John Radcliffe Hospital for further management under the care of the Infectious Diseases Team, inclusive of parenteral anti-malarial therapy if deemed appropriate.

If a participant reaches day 21 following CHMI (C+21) without meeting diagnostic criteria, they shall be started on anti-malarial treatment empirically on this day as described in Section 9.16.

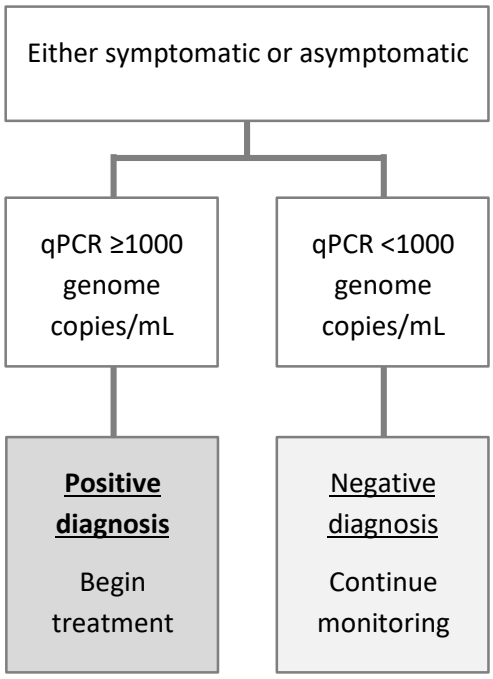


Figure 6: Primary *P. vivax* infection diagnosis and treatment algorithm

9.16. Primary *P. vivax* infection – Medical management

Participants will commence treatment with Malarone (Day of Treatment (DoT)) once a diagnosis of primary *P. vivax* infection is confirmed per the treatment algorithm (Section 9.15). If a participant is unable to take Malarone, an alternative anti-malarial medication, Riamet, will be used to treat blood-stage malaria infection. Participants will not receive Primaquine at this stage (i.e. allowing them to develop relapsing *P. vivax* infection). The monitoring, diagnosis, and treatment of relapse *P. vivax* infections are described in Section 9.17. Participants will be provided with the Patient Information Leaflet of each medication they receive in the study.

Table 8: Overview of malaria medical management

Study event	Primary *P. vivax* infection	Relapse *P. vivax* infections	End of study definitive treatment (C+196)
Drug treatment	*Malarone (or Riamet) ONLY*	*Malarone (or Riamet) ONLY*	*Malarone (or Riamet) AND Primaquine*

9.16.1 Malarone

Malarone contains 100 mg proguanil hydrochloride and 250 mg atovaquone per tablet (see SmPC for Malarone). 4 tablets will be given once daily for 3 days to treat blood-stage *P. vivax* infection. The first and second out of the three doses of Malarone will be directly observed in clinic on Day of Treatment and T+1 respectively. Prior to starting Malarone, participant will be screened for drug interactions and contraindications (including a negative urinary β-hCG). Malarone is generally well tolerated but may cause some side effects, most commonly headache, diarrhoea, nausea, vomiting, stomach pain, dizziness rash, fever, low mood, reduced appetite, cough or sleep disturbance.

Malarone will be given with milk or fatty food to increase absorbance. If a participant vomits within 30 minutes following drug administration, the full dose will be repeated, between 30 and 60 minutes, half of the dose will be given. If vomiting occurs after 60 minutes, re-dosing will not occur.

9.16.2 Riamet

If a participant is unable to take Malarone, an alternative anti-malarial medication, Riamet, will be used to treat blood-stage *P. vivax* infection. Riamet is a combination drug consisting of 20mg artemether and 120mg lumefantrine per tablet (see SmPC for Riamet). The treatment regime will consist of 6 doses of total 80mg artemether/480mg lumefantrine (4 tablets). The first dose, which will be directly observed at treatment initiation, will be followed by additional doses after 8, 24, 36, 48 and 60 hours (window period +/- 1 hour for each dose). The 24-hour dose will also be directly observed.

As Riamet may increase the QT interval, Riamet will not be administered to participants at risk for QT prolongation. Exclusion criteria for this study will include prolonged QT on baseline ECG, a history of long QT syndrome, a family history of congenital QT prolongation or sudden death, cardiac arrhythmias, severe heart disease, and a history of hypokalaemia or hypomagnesaemia. participants will be advised to avoid grapefruit juice whilst taking Riamet as it can affect the bioavailability of artemether.(75)

Prior to starting Riamet, participants will be screened for drug interactions and contraindications. It will not be used for participants who are taking concurrent medications that may prolong QT interval. Participants of childbearing potential on hormonal contraceptive medication will be advised to use an effective additional and/or alternative method of contraception whilst on Riamet treatment as it may decrease the effectiveness of hormonal contraceptives. This should continue until the start of the next menstruation after treatment. A urinary pregnancy test will be performed prior to treatment.

Tablets should be taken together with a fatty meal (a light snack will be provided when doses are observed in clinic). Riamet is generally well tolerated but may cause some side effects. Common side effects include headache, dizziness, abdominal pain and loss of appetite, sleeping problems, palpitations, nausea, vomiting, diarrhoea, pruritus, skin rash, cough, muscle or joint pain and fatigue. Participants will be counselled that certain side effects, for example dizziness, may impact on the performance of skilled tasks such as driving.

If a participant vomits within 30 minutes following drug administration, the full dose will be repeated, between 30 and 60 minutes, half of the dose will be given. If vomiting occurs after 60 minutes, re-dosing will not occur.

9.16.3 Alternative anti-malarial medication

If neither Malarone nor Riamet can be used (e.g. due to side effects) the Investigators may use an alternative appropriate anti-malarial medication informed by current guidelines and best practice. For example, the PvW1 clone of *P. vivax* is sensitive to chloroquine so this would be a reasonable alternative. When oral therapy is not possible (e.g. due to vomiting), participants will be treated parenterally with artesunate until they tolerate oral medication.

9.16.4 Supportive treatment

Provided there are no contraindications, participants will be provided with a course of paracetamol (1g orally up to three times a day) and a course of cyclizine (50 mg orally three times a day) (see SmPC for paracetamol & cyclizine). Participants may be provided with an appropriate, licensed alternative anti-

emetic to cyclizine if they are unable to take cyclizine. Participants will be advised how frequently they can take doses.

9.17. Primary *P. vivax* infection - Treatment follow-up visits (T+1, T+3, T+7)

Participants will be reviewed in clinic on day 1 (T+1) and day 3 (T+3) following initiation of primary *P. vivax* infection treatment (Day of Treatment (DoT)). Participants will attend a further clinic visit on T+7. Thereafter, they will attend a fortnightly "relapse clinic" to monitor for relapsing *P. vivax* infection as outlined in Section 9.18.

At T+3 and T+7, blood samples will be collected for qPCR to ensure clearance of the blood-stage *P. vivax* infection. This may continue 48 hourly until a participant has at least one negative qPCR reading (<20 genome copies/mL) at the discretion of the Investigator**.** Other procedures at these study visits are outlined in Table 5.

9.18. Relapse follow-up period (C+28 – C+196)

Participants will attend a fortnightly "relapse clinic" from 28 days following CHMI (C+28) until 196 days following CHMI (C+196). The purpose of these visits is to monitor for relapsing *P. vivax* infection.

At each routine relapse clinic visit, participants will be questioned regarding the presence of potential malaria symptoms. We will also perform blood-sampling for *P. vivax* parasitaemia by qPCR. Relapse infections will be assessed and managed per the algorithm outlined in Section 9.19.2.

Other procedures required at follow-up visits are outlined in Table 6. These include:

- Assessment of any new medical issues or symptoms by medical history and examination (if required)
- Review of concurrent medication including over the counter medication
- Physical observations including temperature, pulse rate and blood pressure
- Collection of solicited Adverse Events (AEs) i.e. potential malaria symptoms
- Collection of Medically-Attended AEs (MAAEs)
- Collection of any Serious Adverse Events (SAEs)
- Urinary βhCG for participants of childbearing potential
- Blood tests per the study schedule of events (Table 6)

9.19. Relapse *P. vivax* Infection – Assessment pathway and treatment algorithm

The algorithm for assessment and diagnosis of relapse *P. vivax* infection is outlined in Appendix C. More details are outlined below.

9.19.1 Pre-Relapse Assessment (Pre-RAx)

Participants will be provided with a 24hr telephone number for the on-call study doctor. They will be encouraged to contact the study team at any time if they experience symptoms that may represent a relapse *P. vivax* infection.

Although a relapse infection is unlikely to occur in the days immediately following treatment of primary *P. vivax* infection, participants may be assessed by the following processes at any time following T+3 until definitive malaria treatment (C+196).

If a participant reports possible malaria symptoms (either at or between scheduled study visits), an assessment will be conducted by a study clinician. This assessment, termed Pre-Relapse Assessment (Pre-RAx), may be conducted remotely by telephone, and will be used to confirm the reported symptoms.

Table 9: Pre-Relapse Assessment (Pre-RAx) symptom criteria

Classification	Symptom
Primary	Fever (≥ 38°C)
Secondary	Feverishness, chills, rigors, sweats, headache, anorexia, nausea, vomiting, diarrhoea, myalgia, arthralgia, low back pain, fatigue

If a participant reports either one primary symptom or two secondary symptoms (Table 9) during Pre-Relapse Assessment (Pre-RAx), without clear alternative cause, they will be considered symptom criteria positive. They will be invited to attend an in-person Relapse Assessment (RAx) visit.

If a participant reports only one secondary symptom or there is a clear alternative cause for their otherwise positive symptoms (symptom criteria borderline), the Pre-Relapse Assessment (Pre-RAx) will be repeated in 24-48hrs at the discretion or the Investigator.

If a participant does not report any primary or secondary symptoms (symptom criteria negative), they will return to routine relapse clinic follow-up unless there is clinical concern.

9.19.2 Relapse Assessment visit (RAx)

The Relapse Assessment (RAx) visit will be conducted in the following situations:

- Positive malaria symptom criteria on Pre-Relapse Assessment (Pre-RAx)
- Detectable *P. vivax* parasitaemia (qPCR >20 genome copies/mL) on blood sampling from fortnightly relapse clinic visit
- Any clinical concern at the discretion of the Investigator

If blood-sampling from the fortnightly relapse clinic visit demonstrates qPCR 20-100 genome copies/mL, the diagnosis of *P. vivax* relapse infection is considered borderline. The Relapse Assessment (RAx) visit will be used to perform a clinical assessment and repeat the qPCR blood-sample to confirm or refute the diagnosis of relapse *P. vivax* infection. If the repeat qPCR is >100 genome copies/mL, this will confirm a relapse *P. vivax* infection and the participant will be invited to commence blood-stage anti-malarial treatment with Malarone or Riamet at a Relapse Treatment (RTx) visit.

If blood-sampling from the fortnightly relapse clinic visit demonstrates qPCR >100 genome copies/mL the diagnosis of relapse *P. vivax* infection is considered positive. The Relapse Assessment (RAx) visit will be used to perform a clinical assessment AND start blood-stage anti-malarial treatment with Malarone or Riamet (i.e. the Relapse Treatment (RTx) visit will be performed at the same consultation). Blood sampling for qPCR for *P. vivax* parasitaemia will be performed before starting treatment to assess the peak level of parasitaemia associated with the relapse infection (although positive result already confirmed). In this situation, anti-malarial treatment will be started before this repeat qPCR result is returned.

Other study procedures at Relapse Assessment (RAx) visit include:

- Assessment of any new medical issues or symptoms by medical history and examination (if required)
- Review of concurrent medication including over the counter medication
- Physical observations including temperature, pulse rate and blood pressure
- Collection of solicited Adverse Events (AEs)
- Collection of unsolicited AEs
- Collection of any Serious Adverse Events (SAEs)
- Urinary βhCG for participants of childbearing potential
- Blood tests per the study schedule of events (Table 6)

The Investigator may decide to perform an in-person Relapse Assessment (RAx) at any time if there is clinical concern (including after definitive malaria treatment) regardless of symptoms reported by the participant.

9.19.3 Relapse Treatment visits (RTx, RTx+1, RTx+3, RTx+7)

If a participant is diagnosed with a relapse *P. vivax* infection (confirmed by qPCR) they will attend a Relapse Treatment (RTx) visit where they will commence blood-stage anti-malarial treatment with either Malarone or Riamet. They will not receive Primaquine.

Participants will attend follow-up visits on day 1, day 3, and day 7 following initiation of anti-malarial treatment (RTx+1, RTx+3, RTx+7). These Relapse Treatment visits will take place instead of any scheduled relapse clinic visits. After RTx+7, participants will attend the next relapse clinic appointment which is due based on their current timepoint following CHMI (C+ days) and continue fortnightly relapse clinic follow-up thereafter.

If Malarone is used, the first and second out of the three doses of Malarone will be directly observed in clinic at RTx and RTx+1 visits respectively. If Riamet is used, the first dose and 24-hour dose will be directly observed in clinic at RTx and RTx+1 visits respectively.

At RTx+3 and RTx+7, blood samples will be collected for qPCR to ensure clearance of the blood-stage *P. vivax* infection. This may continue 48 hourly until a participant has at least one negative qPCR reading (<20 genome copies/mL) at the discretion of the Investigator**.**

9.20. Relapse *P. vivax* Infection – Medical Management

Participants with a confirmed relapse *P. vivax* infection will receive anti-malarial treatment to treat blood-stage malaria infection with either Malarone or Riamet. They will not receive additional Primaquine.

Malarone will be the first line anti-malarial for relapse *P. vivax* infection. If a participant is unable to take Malarone, Riamet will be used an alternative. Contraindications to Malarone or Riamet treatment will be reviewed prior to administration.

Further details regarding Malarone and Riamet treatment are outlined in Section 9.16. Participants will be provided with supportive medication (paracetamol and anti-emetic) as required as outlined in Section 9.16.

It is expected that participants may experience 2-3 relapse malaria infections during the relapse follow-up period. However, if a participant experiences an excessive number of relapse infections, they may be moved forward in the study schedule to receive definitive malaria treatment (Section 9.21) earlier than the planned timepoint (C+196). This will be at the discretion of the Investigator.

9.21. Definitive treatment

At 6.5 months following CHMI (C+196), participants will undergo definitive anti-malarial treatment with Malarone or Riamet *and* Primaquine to clear any residual blood-stage infection and remaining hypnozoites respectively.

Further details regarding Malarone and Riamet treatment are outlined in Section 9.16. Participants will be reviewed in clinic on day 1 (T+1) and day 3 (T+3) following initiation of treatment.

Participants will commence Primaquine treatment at T+3 following completion of blood-stage anti-malarial treatment. Primaquine treatment will be monitored daily per the schedule of events (Table 7) either by direct observation or by telephone confirmation. Further information about Primaquine treatment is outlined in Section 9.21.1.

Following completion of Primaquine treatment (last dose T+16), participants will be reviewed at one final in-person visit at T+30 (C+226) before being followed up remotely (Section 9.22).

9.21.1 Primaquine

Primaquine is the drug of choice for the clearance of the liver-stage *P. vivax* hypnozoites responsible for future relapse infections (see SmPC for Primaquine). Prior to starting Primaquine, participants will be screened for drug interactions and contraindications to Primaquine, including G6PD deficiency which is associated with a pre-disposition to haemolytic crisis during Primaquine treatment.

Primaquine is contraindicated in pregnancy with a risk of neonatal haemolysis and methaemoglobinaemia in the third trimester. A urine and serum β-HCG test will be performed in participants of childbearing potential at C+196 visit (Malarone or Riamet Day of Treatment) before Primaquine is commenced 3 days later (T+3). All participants of childbearing potential will be required to use highly-effective contraception as described in Section 8.7. Actions to be taken should a participant become pregnant during the study are described in Section 10.5.

A treatment course Primaquine consists of 30mg once daily for 14 days. This starts following completion of blood-stage anti-malarial treatment. At least 3 doses per week will be directly observed in clinic. On the days when a participant is not attending clinic, a telephone call will be conducted to confirm that the participant has taken Primaquine appropriately at home. Participants will be reminded of the potential side effects of Primaquine (primarily nausea and abdominal pain) and provided with the Patient Information Leaflet. They will be advised to take each tablet with food to lessen the gastrointestinal side effects.

As Primaquine is metabolised into redox-active metabolites by CYP2D6, individuals who are unable to metabolise the drug in sufficient quantities appear to be at risk of relapse from *P. vivax* following primaquine treatment (Section 5.5).(30, 31) We shall therefore be assessing our participants for their ability to metabolise Primaquine by genotyping for CYP2D6 at screening. We shall exclude any participant who is not a high metaboliser based on their CYP2D6 genotype.

9.22. Long-term follow-up

Participants will receive an email from the Investigators fortnightly from Month 8 (Day 240) until Month 12 (Day 366) following CHMI. This will provide a link to an electronic questionnaire which will enquire about the presence of any symptoms suggestive of malaria relapse (solicited AEs), any medical intervention sought/received (medically attended AEs), and any changes to medication since last contact. Email follow-up will continue annually thereafter from Year 2 to Year 5 following CHMI. If the participant does not respond, this shall be followed-up by an alternative method such as telephone call, text message or written letter. If they still do not respond, we may contact their next of kin, GP or access electronic health records to check if there have been any changes in circumstance (e.g. moved away, change to health).

In the follow-up emails, participants will be reminded that they are able to contact the study team at any time during this period. In particular, we will strongly encourage participants to contact the study team if they develop symptoms of a possible relapse infection or if they seek medical attention for any unexplained symptoms.

Following the last in-person visit, there are no further travel restrictions. However, participants will be asked to inform the study team of any plans to travel to malaria endemic countries up to 5 years following the malaria challenge. This is so that, in the unlikely event of a malaria infection during this time, the study team can make arrangements to assess the participant (if feasible) and determine if it is an unexpected PvW1 relapse infection (as determined genotyping) or a "new" unrelated malaria infection.

9.23. Unscheduled Visits

Additional visits or procedures may be performed at the discretion of investigators, for example for further medical history and physical examination, additional blood tests or other investigations if clinically relevant.

9.24. Participant samples

Samples will be taken from each participant as outlined in the schedule of study procedures (Section 9.1). The screening samples are for assessment of eligibility. Immunology samples are research samples for analysis as outlined in the Laboratory Analysis Plan.

9.24.1 Clinical Laboratory samples

The processing and analysis of safety blood samples will be carried out at an accredited local clinical laboratory and destroyed following local (NHS) analysis.

- Haematology:
    - Full Blood Count (including haemoglobin, total white blood cells, neutrophil, lymphocyte, eosinophil, platelets)
    - G6PD
    - Haemoglobinopathy screen
- Blood transfusion laboratory:
    - Duffy antigen phenotype
- Biochemistry:
    - Urea and Electrolytes (including sodium, potassium, urea and creatinine)

    - Liver Function Tests (including ALT, ALP, bilirubin, albumin)
    - Serum βHCG (participants of childbearing potential only)
    - Magnesium and cholesterol (screening only)
- Diagnostic serology:
    - Screening tests for Hepatitis B, Hepatitis C and HIV infection (including HBsAg, HCV antibodies, HIV antibodies)
    - EBV and CMV serology
- Other
    - Screening for Primaquine metabolism activity by CYP2D6 genotyping

Additional tests (e.g. hepatitis C qPCR) may be performed, if clinically relevant at the discretion of the medically qualified investigator(s). Tests for Hepatitis B and C are carried out as part of the screening investigations for this trial. Hepatitis B and C are notifiable organisms listed under the UK Health Protection (Notification) Regulations 2010 and, if suspected, will be reported to the UK Healthy Security Agency and the participant's GP. This will be included in the participant information sheet.

9.24.2 *P. vivax* qPCR

It is now well established that nucleic acid amplification-based diagnostics have greater sensitivity than microscopic methods for detecting malaria infection both in natural infection and *P. vivax* CHMI studies. Real-Time quantitative PCR (qPCR) targeting the 18S ribosomal DNA locus will be used as the diagnostic tool for assessment of primary and relapsing *P. vivax* infections.

The only other centre worldwide that routinely performs blood-stage challenge studies with *P. vivax* (QIMR) also uses qPCR as the sole diagnostic tool in CHMI studies and our assay utilises identical primer and probe sequences. In all other aspects the extraction of DNA and qPCR assay is identical to the existing and formally validated *P. falciparum* method. This pan-Plasmodium qPCR is in the process of undergoing a similar formal qualification and work completed so far demonstrates that this is similarly fit for purpose. As examples of qualification parameters, the assay has excellent specificity (within the context of a *P. vivax* only CHMI study), linearity, acceptable accuracy (based on reference samples of microscopically quantified *P. vivax* infected blood), precision and reproducibility (based on several inter-operator tests with acceptable %CV values when assessing prepared samples at the described diagnostic thresholds in >20 replicates).

The assay has an acceptable detection and reliable quantitation limit of >100 genomes per ml, well below reproducible microscopic detection levels. While detection is possible down to approximately 25 genomes per ml (as indicated below) it is less reproducible at this level. The assay is also extremely robust, with both EDTA whole blood samples, extracted DNA and standards remaining stable and giving reproducible qPCR scores at 4°C storage for many days (which is beyond the intended use for real time qPCR follow-up). The range of the qPCR is similar to that of the previously qualified *P. falciparum* assay, with at least a 4 log range from 100 genomes per ml to 1,000,000 for the standards employed (actual range used in assays is from 25 to 1,000,000 genome copies per ml). These criteria assessments are in accordance with the ICH Harmonised Tripartite Guideline Part II: Validation of Analytical Procedures: Methodology (European Medicines Agency, 2006). This is further supported by data from the lab's participation in the UKNEQAS external quality assurance scheme since mid-2018, with 5 rounds of samples assayed in both *P. falciparum* and *P. vivax* assays completed to date, with a 100% success rate.

To date, comparative qPCR and microscopy data are available for two blood-stage *P. vivax* CHMI studies conducted with the PvW1 blood inoculum in Oxford; VAC069A&B (n=11 inclusive of 3 participants who underwent CHMI twice) (NCT03797989) and CHMI of VAC071 Group 1 participants (N=3) (ClinicalTrials.gov Identifier: NCT04009096). Here, the greater sensitivity of qPCR compared to thick film microscopy has been consistently demonstrated with 3/14 participants diagnosed by qPCR prior to microscopic patency and, where infection was microscopically patent (11/14 participants), qPCR detected parasitaemia (>25 genome copies/mL) at least 6 days prior to thick film positivity (as defined as ≥2 asexual forms seen in 200 high-power fields). At the pre-specified qPCR thresholds for diagnosis, 3/14 participants did have a positive thick film result prior to reaching diagnostic criteria (≥5,000 genome copies/mL), however, none of these three participants had experienced symptoms consistent with the clinical threshold for diagnosis at the time of thick smear positivity and were later diagnosed within 1.5 days of microscopic patency, without complication.

Importantly, in the unlikely event of a technical failure with the qPCR machine, a working back-up qPCR machine will be available for use 24/7 if required.

For the purpose of this study, the following thresholds will be used for establishing diagnosis of *P. vivax* primary or relapse infection diagnosis:

- Negative result: qPCR < 20 genome copies per ml or undetectable
- Borderline/uncertain result: qPCR 20-100 genome copies per ml (repeat in 24-48hrs unless clinical concern)
- Positive result: qPCR > 100 genome copies per ml

Following anti-malarial treatment (of either primary or relapse *P. vivax* infection) a negative *P. vivax* qPCR will be required to confirm clearance of parasitaemia. Further blood sampling may continue until the participant has at least one negative qPCR reading (<20 genome copies/mL) at the discretion of the Investigator**.**

9.24.3 Immunology samples

Detail regarding immunology analysis is outlined in the Laboratory Analysis Plan. In brief, we hypothesise that relapsing *P. vivax* infection in humans with a clonal isolate will lead to induction of anti-malarial immune responses that are boosted with each subsequent re-exposure (relapse infection). These may be associated with improved control of recurrent blood-stage parasitaemia.

To assess these hypotheses, we will study the serological response to a panel of *P. vivax* antigens by ELISA and/or antigen array, as well as the cellular responses by flow cytometry. Longitudinal monitoring will assess whether these responses are primed by the primary infection and then boosted by each recurrent relapse/parasite exposure. Purified total IgG will be screened for functional anti-parasitic activity using growth inhibition assays.

Other exploratory immunological tests may be undertaken. This may involve collaboration with other specialist laboratories in the UK, Europe and outside of Europe and therefore the transfer of whole blood, serum, plasma, and peripheral blood mononuclear cells (PBMCs) to these laboratories. Samples would remain pseudo-anonymised. Informed consent for this will be gained from the participants. Assays will be conducted according to local SOPs.

9.24.4 Urine samples

For participants of childbearing potential only, urine will be tested for human chorionic gonadotrophin (βhCG) at screening, before starting anti-malarial treatment and regularly throughout the relapse clinic follow-up period. Urine samples will be destroyed immediately following analysis.

9.24.5 Retention of samples

With the participants' informed consent, any leftover cells, plasma, serum, whole blood (or their purified components) will be registered under University of Oxford HTA licence 12217 and stored indefinitely for future immunological analysis of malaria-specific or vaccine-related responses. This may include human DNA and RNA analysis.

The participant may withdraw permission for future use of specimens at any time. If a participant withdraws their permission for future use of specimens, the Investigator or designee will destroy all known remaining specimens and report this destruction to the participant. This decision will not affect the participant's participation in this protocol or any protocols supported by the Oxford Vaccine Centre. All samples stored will be labelled with the participant's study identification (ID) number, which cannot identify the study participant directly but is linkable to other research databases (e.g., questionnaires, clinical assessments, logbooks) generated by the main study. The subject identification log linking the study participant ID number to the name of the participant will be maintained with access limited to authorised research team members. In the event of samples being requested for separate, further study in the future, only the CI, PIs or study coordinator(s) will have access to the log linking the study participant to the samples. We will not collect duplicate samples.

Only the necessary volume of blood will be collected to address primary, secondary and exploratory objectives of the study. At the completion of the clinical study protocol and the primary and secondary immunology studies described in this protocol, remaining samples will either be destroyed or stored indefinitely. In more detail: for samples held at the University of Oxford, samples from participants who did not provide permission to store them will be discarded after the primary and secondary analyses described in this protocol have been completed. Samples will also be destroyed if the study participants withdraw their permission to store the samples for future studies at any time, and investigator or designee will report the destruction to the participant. If there is no withdrawal all remaining samples will either be destroyed or stored indefinitely (only if the permission to store the samples indefinitely has been obtained from the participants) for future use. Any additional immunological analyses on these samples will be limited to malaria, vaccine or malaria monoclonal antibody development unless specific permission for additional studies is obtained from the relevant ECs. Transfer to another protocol will require approval from the ECs. In the future, other investigators (both at the University of Oxford and outside) may wish to study these samples and/or data. In that case, EC approval must be sought prior to any sharing of samples. Any clinical information shared about the sample with or without participant numbers would similarly require prior EC approval. Blood samples (serum, plasma, whole blood and PBMC) will be stored at the University of Oxford, UK and may be shipped to the collaborative laboratories such as: NIH/NIAID Laboratory of Malaria and Vector Research, Malaria Immunology Section, GIA Reference Centre, 12735 Twinbrook Parkway, Twinbrook III, Room 3W-13, Rockville, MD 20852, USA.

At the University of Oxford laboratories, freezer and refrigerator temperatures are continuously recorded by a data logger.

9.25. Early Discontinuation/Withdrawal of Participants

Each participant can exercise their right to withdraw from the study at any time without giving a reason. In addition, the investigator may withdraw a participant from the study at any time for the following, although not exhaustive, reasons:

- The investigator considers it necessary for participant safety.
- Significant non-compliance with study requirements.
- The participant is lost to follow-up.

In circumstances pertaining to the safety of the participant, the DSMC chair, DSMC committee or Investigator may choose to discontinue further specific study procedures for an individual participant. However, participants should otherwise continue to attend the follow-up visit schedule and follow-up procedures unless they withdraw consent for this. Such circumstances may include the following non-exhaustive reasons:

- Pregnancy (further details on management of participants who become pregnant are provided in Section 10.5).
- An adverse event which requires discontinuation of the study procedures or results in an inability to continue to comply with study procedures.
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening).

Withdrawal from the study will not result in exclusion of existing data generated by the participant from analysis. Participants can request that their samples are destroyed at any point during or after the study (although data that has already been generated from samples that have been analysed up to that point will be retained). The reason for withdrawal, if given, will be recorded in the eCRF.

If a participant wishes to withdraw from the study, then a complete, appropriate, curative course of anti-malarial therapy (including Primaquine) must be completed. The importance of this will be emphasised to participants at screening.

9.26. Definition of End of Study

The end of the trial will be complete when all assays providing data for primary and secondary endpoints have been completed.

10. SAFETY CONSIDERATIONS

10.1. Safety measures for conduct of a CHMI

Participant safety is of paramount importance. The following measures are in place to safeguard participant safety:

i) Participants will only be enrolled in the study if Investigators are satisfied that they fulfil stringent inclusion and exclusion criteria.

ii) Participants' understanding of the trial information will be tested by means of a questionnaire at screening. This provides further confidence that fully informed consent has been obtained.

iii) If the participant does not have their own mobile telephone they will be issued with one for the duration of the study and counselled about the importance of keeping it switched on or checking the messages regularly.

iv) Before CHMI, full contact details for each subject will be documented, including home address and mobile telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the participants are easily contactable. Home and work landline telephone numbers where available and next-of-kin address and telephone numbers will also be documented. Subjects must also provide the Investigators with the name and 24-hour telephone number of a close friend, relative or housemate who lives nearby and will be kept informed of their whereabouts for the duration of the study.

v) The study team will accompany participants to RUMC, Nijmegen, Netherlands and will be responsible for their safety and well-being at each stage of the malaria challenge, including the journey to/from Nijmegen and while staying in accommodation.

vi) The CHMI will be conducted at a world-leading malaria research insectary at RUMC which has extensive experience in conducting sporozoite malaria challenge studies administered by mosquito-bite.

vii) The participants will be provided with a MedicAlert card containing contact details for the study team and brief details of the study including the optimal treatment for *P. vivax* malaria.

viii) Two doses of Malarone or Riamet will be observed for each participant by the study team during treatment of primary or relapsing *P. vivax* infection.

ix) Appropriate Primaquine treatment will be monitored daily during the treatment course, either by direct observation or remote telephone call.

x) Participants will be counselled that should they fail to return for treatment having been infected with *P. vivax* they could become very unwell and potentially die. They will be instructed to remain in Oxfordshire (or surrounding area) following malaria challenge (after return from the Netherlands) until completion of treatment of primary *P. vivax* infection. They will be informed that should they fail to attend a scheduled clinic visit following CHMI, and be uncontactable by telephone, their nominated contact and/or next of kin will be contacted. If their whereabouts cannot be determined, the police and media may be informed in order to start a search.

xi) Participants will be able to contact a medically qualified member of the study team 24 hours a day throughout the study period and will be instructed to contact the Investigator immediately should they manifest any signs or symptoms they perceive as serious.

10.2. Safety measures during relapse follow-up period

The following additional safety measures are in place to safeguard the safety of participants in the relapse follow-up period. During this time, we expect participants to develop relapse *P. vivax* infections. Therefore creating conditions so that these occur in a safe manner is of paramount importance.

i) Participants will be encouraged to contact the study team if they experience symptoms suggestive of relapse malaria infection. There will be a 24/7 on-call study doctor throughout the relapse clinic follow-up period.

ii) Participants will be seen in person at a fortnightly clinic throughout the relapse clinic follow-up period. These visits will involve clinical assessment and monitoring or parasitaemia by qPCR.

iii) Participants will be required to remain within travelling distance of Oxfordshire (or surrounding area) following treatment of primary *P. vivax* infection until completion of Primaquine treatment (month 7). This is to ensure that participants will be able to seek prompt treatment of relapse *P. vivax* infections. A degree of discretion on the part of the Investigator will be required as it is not practical nor ethical to restrict participants to the immediate radius of CCVTM for a 6-month period. For example, short trips to other areas in the UK with a named individual (who is aware of

their trial participation and has contact details of the study team) may be permitted. The absolute necessity during this time is that participants remain on the UK mainland within 1-2 hours of a NHS hospital in order to ensure participants are able to access anti-malarial treatment if required in an emergency.

iv) Participants must be contactable by telephone throughout the relapse follow-up period.

v) We will counsel participants of the importance of not leaving the country. This would not only pose risks to themselves, but also risks introducing or spreading *P. vivax* malaria in countries with a suitable mosquito population.

vi) The Infectious Diseases Team, Oxford University Trust, will be informed of this study

vii) It is expected that participants may experience 2-3 relapse malaria infections during the relapse follow-up period.(31, 74) However, if a participant experiences an excessive number of relapse infections, they may be moved forward in the study schedule to receive definitive malaria treatment (Section 9.21) earlier than the planned timepoint (C+196). This will be at the discretion of the Investigator.

10.3. Procedure if a participant goes missing

In the unlikely event that a participant is uncontactable following CHMI and before completion of Primaquine treatment, the following stakeholders will be informed.

i) All Investigators.
ii) The participant's nominated contact and next of kin.
iii) The trial Sponsor.
iv) The local safety committee.
v) The ethics committee(s).
vi) Relevant hospital trust R&D departments.
vii) The local police department.
viii) Local Accident and Emergency and Infectious Diseases departments.

Active efforts will be made to locate the participant by the police. While all parties will aim to preserve the participant's confidentiality, if necessary, details of the participant's identity and participation in the study may be passed to the national media in order to help locate the missing individual. Participants will be informed of this during screening.

10.4. Criteria for Transfer to NHS Hospital Care

If any of the following criteria are met, admission under the care of the Infectious Diseases Team, Oxford University Hospitals NHS Foundation Trust will be considered:

- Failure of symptoms to improve within 48 hours of starting anti-malarial therapy.
- Unable to tolerate oral anti-malarial therapy.
- Dehydration requiring intravenous fluid therapy.
- Signs or symptoms suggestive of pulmonary oedema.
- Signs or symptoms of neurological dysfunction including altered consciousness.
- Signs, symptoms or laboratory evidence of significant renal dysfunction.
- Unanticipated concern about the subject's home circumstances.
- Any other significant finding which the Investigators feel warrants inpatient admission

Ultimately, the decision regarding admission will be taken by the Investigators in conjunction with the Infectious Diseases Consultant on call.

10.5. Pregnancy

P. vivax infection (primary or relapse) can pose a serious risk to the health of a pregnant mother and the unborn fetus. In addition, Primaquine treatment is contraindicated in pregnancy.

Pregnancy, or intention to become pregnant, is therefore an exclusion criterion for participation in this study and participants of childbearing potential are required to use highly-effective contraception as described in Section 8.7. A serum β-HCG test will be performed in participants of childbearing potential 2 days prior to CHMI (at C-2). A positive result would be a contraindication to CHMI.

We will conduct regular urinary β-hCG tests throughout the follow-up period (and serum β-hCG prior to initiation of Primaquine treatment) to identify early any pregnancy that occurs following CHMI and prior to completion of Primaquine treatment.

Any participant who becomes pregnant during the trial will be promptly discussed with the DSMC and, if necessary, the Infectious Diseases consultant (+/- Obstetrics consultant) on call in OUH NHS Trust. The most appropriate course of action will be informed by these specialists, current guidelines and most up to date evidence. The recommended treatment may depend on individual circumstances and a balance of potential risks and benefits (e.g. risk of treatment with Primaquine vs risk of relapsing *P. vivax* infection to mother and fetus). Although not prescriptive for this scenario, as an example, the CDC and UK guidelines recommend chloroquine chemoprophylaxis for the duration of pregnancy in pregnant mothers with *P. vivax* infection. After delivery, Primaquine treatment may be initiated but, for woman who are breastfeeding, the infant should be tested for G6PD deficiency beforehand. (26, 76)

Any participant who becomes pregnant will be followed up until the pregnancy outcome, with the participant's permission. Depending on the timings of pregnancy outcome relative to planned study follow-up timepoints, this may involve additional telephone consultations and/or performing routine follow-up visits by telephone. We will not routinely perform venepuncture on such participants.

11. SAFETY REPORTING

Safety will be assessed by recording the frequency, incidence and nature of AEs and SAEs that arise following CHMI.

11.1. Adverse Events (AEs)

An Adverse Event (AE) is any untoward medical occurrence in a participant, including a dosing error, which may occur during or after administration of a study intervention (i.e. CHMI in this study). It does not necessarily have a causal relationship with the intervention. An untoward medical occurrence may be an unintended symptom, sign, disease, or abnormal laboratory finding. AEs will be recorded according to the schedule of study procedures outlined in Section 9.1.

11.2. Solicited Adverse Events

Solicited Adverse Events (Solicited AEs) refer to the foreseeable symptoms, signs and laboratory findings which may occur following a study intervention (i.e. sporozoite CHMI). This includes i) following mosquito bites, or ii) following *P. vivax* infection (either primary or relapse), or iii) following anti-malarial or supportive medications provided by the study team.

Solicited adverse events related to mosquito bites are itch, redness, swelling and warmth. These will be recorded from CHMI to Day of Treatment of primary malaria infection. These will be graded according to Table 11.

Solicited adverse events related to malaria infection are outlined in Table 10. Participants will be actively questioned regarding the presence of symptoms related to malaria infection (Table 10) at in person visits following CHMI (C+7 – DoT). This will continue throughout the relapse follow-up period until C+196, and by email from month 8 – year 5.

Solicited adverse events related to Malarone, Riamet, Primaquine, paracetamol or cyclizine, are listed as undesirable effects in the Summary of Product Characteristics (SmPCs) for these medications. These will be solicited while the participant is taking this medication. Participants will be asked if there have been any changes to their health but will not be actively questioned for specific symptoms related to these medications.

Table 10: Solicited AEs relating to malaria infection

Symptoms	Signs	Laboratory findings
Feverishness Chills Rigor Sweats Headache Anorexia Nausea Vomiting Diarrhoea Myalgia Arthralgia Low back pain Fatigue	Fever Tachycardia Hypotension	Lymphopenia Thrombocytopenia

11.3. Serious Adverse Events (SAEs) and Medically-Attended Adverse Events (MAAEs)

A Serious Adverse Event (SAE) is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other important medical events may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

A Medically-Attended Adverse Event (MAAE) refers to any untoward medical occurrence that led to assessment by a healthcare provider.

11.4. Reporting Procedures for SAEs and MAAEs

SAEs will be reported to an internal safety group (members of the study team) within 24 hours of the Investigators being aware of their occurrence, as described in the SOP OVC005. This safety group includes the Chief Investigator, who acts on behalf of the Sponsor for notification of SAEs. The DSMC will be notified of SAEs deemed possibly, probably or definitely related to study interventions. SAEs will not normally be reported to the ethical committee(s) unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial participants, at the discretion of the Chief Investigator and/or DSMC.

SAEs will be collected from point of consent until the end of a participant's participation in the study. MAAEs will be collected from CHMI until the end of a participant's participation in the study . SAEs will be recorded on the SAE eCRF and will be reported by completing the electronic SAE form (or paper back up) within 24 hours of the site being notified of the event. Additional information received for an SAE (follow up or corrections to the original report) will be detailed on a new SAE form, indicating 'update'. All SAEs will be followed up until resolution, the event is considered stable or until a non-study causality is assigned.

11.5. Severity grading of AEs

The severity of adverse events will be assessed by the Investigators according to the scales in Tables 11-12.

Table 11: Severity grading of AEs

Grade	Description
GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; may require medical intervention/therapy

Table 12: Severity grading for clinically significant physical observations

Physical Observations	Grade 1	Grade 2	Grade 3
Tachycardia – beats per min*	101-115	116-130	>130
Hypotension (systolic) mm Hg	85-89	80-84	<80
Hypertension (systolic) mm Hg**	141-159	160-179	>180
Hypertension (diastolic) mm Hg**	91-99	100-109	>110
Fever °C	37.6 – 38.0	38.1-39.0	>39.0

*Only clinically significant abnormal physical observations will undergo severity grading. All observations should be measured at rest. *Only applies when resting heart rate is between 60 and 100 beats per minute. Use clinical judgement when characterising bradycardia in some healthy subject populations (e.g. conditioned athletes). **Systolic or diastolic hypertension may only be confirmed as clinically significant (and therefore an AE) if persistently present when observations are repeated (i.e. isolated measurements of hypertension are not clinically significant).*

11.6. Severity grading for laboratory AEs

Severity grading for laboratory AEs are dependent on the OUH laboratory's reference and will be graded according to the scales in Appendix B. These ranges will be based on United States Food and Drug Administration (FDA) guidance relative to local laboratory reference ranges.(77)

11.7. Causality assessment of AEs

For every unsolicited AE, an assessment of the relationship of the AE to the study intervention will be undertaken by the CI-delegated clinician. The "study intervention" is considered sporozoite *P. vivax* PvW1 CHMI and, for the purpose of AE causality assessment, includes i) mosquito bites, ii) *P. vivax* infection (either primary or relapse), and iii) anti-malarial or supportive medications provided by the study team. The relationship of the adverse event to the study intervention will be categorised as unrelated, unlikely to be related, possibly related, probably related or definitely related (Table 14). An intervention-related AE refers to an AE for which there is a possible, probable, or definite relationship to the study intervention. The delegated clinician will use clinical judgment to determine the relationship. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship will be considered and investigated. Causality assessment will take place at the final safety analysis, except for SAEs, which should be assigned by the reporting Investigator.

Table 13: Guidelines for assessing the relationship of study intervention to an AE

0	No Relationship	No temporal relationship to study intervention ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to CHMI
1	Unlikely	Unlikely temporal relationship to study intervention ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical pattern of response to CHMI
2	Possible	Reasonable temporal relationship to study intervention; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that known to occur following CHMI
3	Probable	Reasonable temporal relationship to study intervention; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response to that known to occur following CHMI
4	Definite	Reasonable temporal relationship to study intervention; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response to that known to occur following CHMI

12. STATISTICS AND ANALYSIS

This is a proof on concept experimental CHMI study to assess the safety and feasibility of relapsing *P. vivax* PvW1 infection after experimental sporozoite CHMI administered by mosquito bite. Five participants will undergo sporozoite CHMI. This number was chosen to ensure there was a reasonable likelihood of successfully inducing relapsing *P. vivax* infection, accounting for risk of failure of sporozoite CHMI to cause primary *P. vivax* infection, latency of first relapse (in some participants) being greater than 6 months, and withdrawal of participants. This is the first study of its kind to be performed and the outcomes are descriptive. All available data will be used in the analyses, performed by suitably trained and delegated individuals, and there will be no imputation for missing data.

13. DATA MANAGEMENT

The data management aspects of the study are summarised here, with details fully described in the Data Management Plan.

Each study participant will have a unique participant ID which will be allocated at the time of the screening visit. Names or identifying details are not included in any electronic file, containing study data. Apart from clinical safety blood samples which may be sent to local clinical laboratories and follow local sample labelling requirements, samples sent to laboratories for processing will be identified by trial number and participant number only.

13.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital or GP records (from which medical history and previous and concurrent medication may be summarised into the CRF), laboratory and vaccination/pharmacy records, diaries, ultrasound images, and correspondence.

In this study, eCRF entries will be considered source data where it is the site of the original recording (e.g., there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent and the participant contact sheet, the participant will be referred to by the trial participant number, not by name.

13.2. Access to Data

Direct access will be granted to authorised representatives from (or appointed by) the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits, and inspections.

13.3. Data Recording and Record Keeping

All study data will be recorded directly into eCRFs within Electronic Data Capture (EDC) system (e.g., REDCap, or similar), or onto a paper source document for later entry into the EDC system if direct entry is not available. Any additional information that needs recording but is not relevant for the eCRF (such as signed consent forms) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which

are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personnel. The servers are in a physically secure location in Europe, and data are backed up on secure servers operated by the University of Oxford IT Services, physically located in Europe. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. Weekly backup tapes are stored offsite. The servers provide a stable, secure, well-maintained, and high-capacity data storage environment. REDCap is a widely used, powerful, reliable, well-supported system. Access to the study's database will be restricted to the members of the study team by username and password.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (*e.g.,* requesting to be informed of other trials), participant's personal details will not be used to contact them other than in exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

14. QUALITY ASSURANCE PROCEDURES

14.1. Investigator procedures

Approved standard operating procedures (SOPs) will be used at all clinical and laboratory sites.

14.2. Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities. Approved and relevant SOPs will be used at all clinical and laboratory sites.

14.3. Study monitoring

Monitoring will be performed according to GCP by OVG. Following written SOPs, the monitors will verify that the study is conducted, and data are generated, documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements. Trial site(s) will provide direct access to all trial-related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

14.4. Data and Safety Monitoring Committee (DSMC)

The DSMC will be chaired by Dr Ruth Payne (Consultant Microbiologist and Senior Clinical Lecturer) and will consist of two independent clinicians and one independent statistician.

The DSMC is independent and will review safety data throughout the study according to the DSMC Charter. The specific role of the committee will be as follows:

1. Monitor safety data with regards to relapses
2. Unscheduled reviews on request of the study team
3. At the end of the study, review the study's progress including data on main outcomes and safety.

When required, the DSMC will make recommendations to the study investigators on whether there are any ethical or safety reasons why the study should not continue. A summary of all AEs and SAEs to date will be provided to the DSMC on request.

The outcome of each DSMC review will be communicated directly to the study investigators and documentation of all reviews will be kept in the TMF.

The Chair of the DSMC will also be contacted for advice where the Chief Investigator feels independent advice or review is required.

15. PROTOCOL DEVIATIONS

Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file. Each deviation will be assessed as to its impact on participant safety and study conduct. Significant deviations will be listed in the end of study report.

16. SERIOUS BREACHES

A "serious breach" is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

17. ETHICAL AND REGULATORY CONSIDERATIONS

17.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

17.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

17.3. Approvals

Following Sponsor approval the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

17.5. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database. Results will be uploaded to the ISRCTN Database within 12 months of the end of trial (as declared by the CI or their delegate). Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

17.6. Participant Confidentiality

The trial staff will ensure that the participants' confidentiality is maintained. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

17.7. Participant Financial Compensation

The compensation for the screening visit is £110. We will be providing additional reimbursement to cover the time and inconvenience of the 3-day journey to the Netherlands. This will be £450. Participants will be compensated for the time and inconvenience of other study visits as per below.

- Travel expenses	£30 per visit
- Inconvenience of blood tests:	£20 per blood donation
- Time required for visit:	£40 per hour
- Compensation for time off work:	£150 per outpatient days*

*(max. 3 days)

Additional reimbursement for unscheduled visits at £90 per visit will be provided. This will not be given unless an unscheduled visit occurs.

The total amount of compensation for an individual participant will depend on the actual number of visits attended. This in turn depends on i) date of diagnosis for primary *P. vivax* infection ii) number of relapse infections and iii) whether any repeat or additional visits were necessary. The estimated total compensation for participation in the study (assuming 2 relapse infections and attendance at all study visits) is approximately £5,270. In event of zero relapse infections, the estimated compensation (assuming attendance at all study visits) is approximately £4,630.

If a participant withdraws consent for continued participation in the trial or is withdrawn for any other reason, they will still be compensated for any trial visits they attended.

18. FINANCE AND INSURANCE

18.1. Funding

BIO-006 study is co-funded from European Union Horizon Europe programme and from UK Research and Innovation (UKRI) via OptiViVax consortium, an international consortium across nine academic institutions and industry partners with the goal of developing the necessary tools to accelerate vaccine development against *P. vivax* infection.

18.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). An additional policy or a separate local insurance policy will be arranged to cover study activities at RUMC.

18.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

19. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the International Committee of Medical Journal Editors guidelines and other contributors will be acknowledged. Data from the study may also be used as part of a thesis for a PhD or MD.

A lay summary of the study results may be provided to participants at the end of the study.

20. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The protection and exploitation of any new IP is managed by the University's technology transfer office, Oxford University Innovations.

21. ARCHIVING

Study data will be stored electronically on a secure server, and paper notes will be kept in a secure location at the study site(s) or as outlined in local SOP's. All essential documents, which includes research data and identifiable information, will be retained for a minimum of 5 years after the study has finished.. Participants' bank details will be stored for a minimum of 7 years in line with the site financial policy. Participants who complete online screening or telephone screening only (before informed consent) will not have data kept beyond the end of the trial.

22. REFERENCES

1. Battle KE, Lucas TCD, Nguyen M, Howes RE, Nandi AK, Twohig KA, et al. Mapping the global endemicity and clinical burden of Plasmodium vivax, 2000-17: a spatial and temporal modelling study. Lancet. 2019;394(10195):332-43.

2. WHO Malaria Report 2023.

3. Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA. Key gaps in the knowledge of Plasmodium vivax, a neglected human malaria parasite. Lancet Infect Dis. 2009;9.

4. Wells TN, Burrows JN, Baird JK. Targeting the hypnozoite reservoir of Plasmodium vivax: the hidden obstacle to malaria elimination. Trends Parasitol. 2010;26(3):145-51.

5. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax Malaria Neglected and Not Benign. Am J Trop Med Hyg. 2007;77:79–87.

6. Baird JK. Neglect of Plasmodium vivax malaria. Trends Parasitol. 2007;23(11):533-9.

7. WHO. WHO guidelines for malaria. 16 October 2023.

8. Barcus MJ, Basri H, Picarima H, Manyakori C, Sekartuti., Elyazar I, et al. Demographic risk factors for severe and fatal Vivax and Falciparum malaria among hospital admissions in Northeastern Indonesian Papua. Am J Trop Med Hyg.77(5):984–91.

9. Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, Alpers MP, Muller I. Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. PLoS Med. 2008;5(6):e127.

10. Nosten F, McGready R, Simpson JA, Thwai KL, Balkan S, Cho T, et al. Effects of Plasmodium vivax malaria in pregnancy. Lancet. 1999;354(9178):546-9.

11. McGready R, Lee SJ, Wiladphaingern J, Ashley EA, Rijken MJ, Boel M, et al. Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. Lancet Infect Dis. 2012;12(5):388-96.

12. Mendis K, Sina BJ, Marchesini P, CARTER R. The Neglected Burden of Plasmodium vivax Malaria. Am J Trop Med Hyg. 2001;64((1,2)S):97–106.

13. Konradsen F, van der Hoek W, Amerasinghe PH, Amerasinghe FP, Fonseka KT. Household responses to malaria and their cost a study from rural Sri Lanka. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1997;91:127-30.

14. Commons RJ, Simpson JA, Watson J, White NJ, Price RN. Estimating the Proportion of Plasmodium vivax Recurrences Caused by Relapse: A Systematic Review and Meta-Analysis. Am J Trop Med Hyg. 2020;103(3):1094-9.

15. Adekunle AI, Pinkevych M, McGready R, Luxemburger C, White LJ, Nosten F, et al. Modeling the dynamics of Plasmodium vivax infection and hypnozoite reactivation in vivo. PLoS Negl Trop Dis. 2015;9(3):e0003595.

16. Maneerattanasak S, Gosi P, Krudsood S, Chimma P, Tongshoob J, Mahakunkijcharoen Y, et al. Molecular and immunological analyses of confirmed Plasmodium vivax relapse episodes. Malar J. 2017;16(1):228.

17. Doherty JF, Day JH, Warhurst DC, Chiodini Pl. Treatment of Plasmodium vivax - time for a change. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1997;91:76.

18. Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, et al. Relapses of Plasmodium vivax infection usually result from activation of heterologous hypnozoites. J Infect Dis. 2007;195(7):927-33.

19. Kitchener SJ, Auliff AM, Rieckmann KH. Malaria in the Australian Defence Force during and after participation in the International Force in East Timor (INTERFET). Med J Aust. 2000;173(11-12):583-5.

20. Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of Plasmodium vivax infection result from clonal hypnozoites activated at predetermined intervals. J Infect Dis. 2007;195(7):934-41.

21. Smoak BL, DeFraites RF, Magill AJ, Kain KC, Wellde BT. Plasmodium vivax infections in US Army Troops - Failure of Primaquine to prevent relapse in stuides from Somalia. Am J Trop Med Hyg. 1997;56(2):231-4.

22. Battle KE, Karhunen MS, Bhatt S, Gething PW, Howes RE, Golding N, et al. Geographical variation in Plasmodium vivax relapse. Malaria Journal. 2014;13.

23. Vale N, Moreira R, Gomes P. Primaquine revisited six decades after its discovery. Eur J Med Chem. 2009;44(3):937-53.

24. Tan KR, Hwang J. Tafenoquine receives regulatory approval in USA for prophylaxis of malaria and radical cure of Plasmodium vivax. J Travel Med. 2018;25(1).

25. Llanos-Cuentas A, Lacerda MVG, Hien TT, Velez ID, Namaik-Larp C, Chu CS, et al. Tafenoquine versus Primaquine to Prevent Relapse of Plasmodium vivax Malaria. N Engl J Med. 2019;380(3):229-41.

26. Lalloo DG, Shingadia D, Bell DJ, Beeching NJ, Whitty CJM, Chiodini PL, Travellers PHEACoMPiU. UK malaria treatment guidelines 2016. J Infect. 2016;72(6):635-49.

27. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371(9606):64-74.

28. Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, et al. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. PLoS Med. 2012;9(11):e1001339.

29. Commons RJ, Rajasekhar M, Edler P, Abreha T, Awab GR, Baird JK, et al. Effect of primaquine dose on the risk of recurrence in patients with uncomplicated Plasmodium vivax: a systematic review and individual patient data meta-analysis. Lancet Infect Dis. 2024;24(2):172-83.

30. Pybus BS, Marcisin SR, Jin X, Deye G, Sousa JC, Li Q, et al. The metabolism of primaquine to its active metabolite is dependent on CYP 2D6. Malaria Journal. 2013;12(212).

31. Bennett JW, Yadava A, Tosh D, Sattabongkot J, Komisar J, Ware LA, et al. Phase 1/2a Trial of Plasmodium vivax Malaria Vaccine Candidate VMP001/AS01B in Malaria-Naive Adults: Safety, Immunogenicity, and Efficacy. PLoS Negl Trop Dis. 2016;10(2):e0004423.

32. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther. 2008;83(2):234-42.

33. Payne RO, Griffin PM, McCarthy JS, Draper SJ. Plasmodium vivax Controlled Human Malaria Infection - Progress and Prospects. Trends Parasitol. 2017;33(2):141-50.

34. Voorberg-van der Wel AM, Zeeman AM, Nieuwenhuis IG, van der Werff NM, Klooster EJ, Klop O, et al. A dual fluorescent Plasmodium cynomolgi reporter line reveals in vitro malaria hypnozoite reactivation. Commun Biol. 2020;3:7.

35. Gural N, Mancio-Silva L, Miller AB, Galstian A, Butty VL, Levine SS, et al. In Vitro Culture, Drug Sensitivity, and Transcriptome of Plasmodium Vivax Hypnozoites. Cell Host Microbe. 2018;23(3):395-406 e4.

36. Schafer C, Dambrauskas N, Reynolds LM, Trakhimets O, Raappana A, Flannery EL, et al. Partial protection against P. vivax infection diminishes hypnozoite burden and blood-stage relapses. Cell Host Microbe. 2021;29(5):752-6 e4.

37. Sciences TAoM. Controlled Human Infection Model Studies. In: HIC-Vac, MRC, Wellcome, editors. 2018.

38. Moxon. Microbial Challenge Studies of Human Volunteers. The Academy of Medical

Sciences. 2005.

39. Lees R. Treatment of General Paralyss of the Insane by Induced Malaria: Note on Fifty Cases. Br Med J. 1931;22(3685):336-9.

40. Snounou G, Perignon JL. Malariotherapy--insanity at the service of malariology. Adv Parasitol. 2013;81:223-55.

41. Alving A, Craige B, Pullman T, Whorton CM, Jones R, Eichelberger L. Procedures Used at Stateville Penitentiary for the Testing of Potential Antimalaria Agents. Journal of Clinical Investigation. 1946;27(3):2.

42. Riekmann KH, Beaudoin RL, Cassells JS, Sell KW. Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. Bulletin of the World Helath Organization. 1979;57:261-5.

43. Comfort N. The prisoner as model organism: malaria research at Stateville Penitentiary. Stud Hist Philos Biol Biomed Sci. 2009;40(3):190-203.

44. Miller LH, Mason SJ, Clyde DF, McGuiness MH. The resistance factor to Plasmodium vivax in

blacks. The Duffy-blood-group genotype, FyFy. N Engl J Med. 1976;295(6).

45. Trager W, Jensen J. Human Malaria Parasites in Continuous Culture. Science. 1976;193(4254):673-5.

46. Ifediba T, Vanderberg JP. Complete in vitro maturation of Plasmodium falciparum gametocytes. Nature. 1981;294(5839):364-6.

47. Campbell CC, Collins WE, Nguyen-Dinh P, Barber A, Broderson JR. Plasmodium falciparum gametocytes from culture in vitro develop to sporozoites that are infectious to primates. Science. 1982;10(4564):1048-50.

48. Chulay JD, Schneider I, Cosgriff TM, Hoffman SL, Ballou WR, Quakyi IA, et al. Malaria Transmitted to Humans by Mosquitoes Infected from Cultured Plasmodium falciparum. Am J Trop Med Hyg. 1986;35(1):66-8.

49. Ballou WR, Hoffman SL, Sherwood JA, Hollingdale MR, Neva FA, Hockmeyer WT, et al. Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine. Lancet. 1987;1(8545):1277-81.

50. Herrington DA, Clyde DF, Losonsky G, Cortesia M, Murphy JR, Davis J, et al. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against Plasmodium falciparum sporozoites. Nature. 1987;328:257-9.

51. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. J Infect Dis. 2009;200(3):337-46.

52. WHO. WHO recommends groundbreaking malaria vaccine for children at risk 2021 [Available from: https://www.who.int/news/item/06-10-2021-who-recommends-groundbreaking-malaria-vaccine-for-children-at-risk.

53. Targett GA, Moorthy VS, Brown GV. Malaria vaccine research and development: the role of

the WHO MALVAC committee. Malar J. 2013;12(1).

54. Roestenberg M, Hoogerwerf MA, Ferreira DM, Mordmuller B, Yazdanbakhsh M. Experimental infection of human volunteers. Lancet Infect Dis. 2018;18(10):e312-e22.

55. Epstein JE, Rao S, Williams F, Freilich D, Luke T, Sedegah M, et al. Safety and clinical outcome of experimental challenge of human volunteers with Plasmodium falciparum-infected mosquitoes: an update. J Infect Dis. 2007;196(1):145-54.

56. Verhage DF, Telgt DSC, Bousema JT, Hermsen CC, van Gemert GJA, van der Meer JWM, Sauerwein RW. Clinical outcome of experimental human malaria induced by Plasmodium falciparum-infected mosquitoes. Neth J Med. 2005;63(2):52-8.

57. Arevalo-Herrera M, Vasquez-Jimenez JM, Lopez-Perez M, Vallejo AF, Amado-Garavito AB, Cespedes N, et al. Protective Efficacy of Plasmodium vivax Radiation-Attenuated Sporozoites in Colombian Volunteers: A Randomized Controlled Trial. PLoS Negl Trop Dis. 2016;10(10):e0005070.

58. Odedra A, Webb L, Marquart L, Britton LJ, Chalon S, Moehrle JJ, et al. Liver Function Test Abnormalities in Experimental and Clinical Plasmodium vivax Infection. Am J Trop Med Hyg. 2020;103(5):1910-7.

59. Reuling IJ, de Jong GM, Yap XZ, Asghar M, Walk J, van de Schans LA, et al. Liver Injury in Uncomplicated Malaria is an Overlooked Phenomenon: An Observational Study. EBioMedicine. 2018;36:131-9.

60. Sandoval DM, Bach F, Nahrendorf W, Ivens A, Mazurczyk M, Themistocleous Y, et al. 2021.

61. Chughlay MF, Akakpo S, Odedra A, Csermak-Renner K, Djeriou E, Winnips C, et al. Liver Enzyme Elevations in Plasmodium falciparum Volunteer Infection Studies: Findings and Recommendations. Am J Trop Med Hyg. 2020;103(1):378-93.

62. Azasi Y, Lindergard G, Ghumra A, Mu J, Miller LH, Rowe JA. Infected erythrocytes expressing DC13 PfEMP1 differ from recombinant proteins in EPCR-binding function. Proc Natl Acad Sci U S A. 2018;115(5):1063-8.

63. Van Meer MPA, Bastiaens GJH, Boulaksil M, De Mast Q, Gunasekera A, Hoffman SL, et al. Idiopathic acute myocarditis during treatment for controlled human malaria infection: a case report. Malar J.13(1):38.

64. Arevalo-Herrera M, Forero-Pena DA, Rubiano K, Gomez-Hincapie J, Martinez NL, Lopez-Perez M, et al. Plasmodium vivax sporozoite challenge in malaria-naive and semi-immune Colombian volunteers. PLoS One. 2014;9(6):e99754.

65. Herrera S, Fernandez O, Manzano MR, Murrain B, Vergara J, Blanco P, et al. Successful sporozoite challenge model in human volunteers with Plasmodium vivax strain derived from human donors. Am J Trop Med Hyg. 2009;81(5):740-6.

66. Herrera S, Solarte Y, Jordan-Villegas A, Echavarria JF, Rocha L, Palacios R, et al. Consistent safety and infectivity in sporozoite challenge model of Plasmodium vivax in malaria-naive human volunteers. Am J Trop Med Hyg. 2011;84(2 Suppl):4-11.

67. Minassian AM, Themistocleous Y, Silk SE, Barrett JR, Kemp A, Quinkert D, et al. Controlled human malaria infection with a clone of Plasmodium vivax with high-quality genome assembly. JCI Insight. 2021;6(23).

68. Arevalo-Herrera M, Gaitan X, Larmat-Delgado M, Caicedo MA, Herrera SM, Henao-Giraldo J, et al. Randomized clinical trial to assess the protective efficacy of a Plasmodium vivax CS synthetic vaccine. Nat Commun. 2022;13(1):1603.

69. Hou MM, Barrett JR, Themistocleous Y, Rawlinson TA, Diouf A, Martinez FJ. Vaccination with Plasmodium vivax Duffy-binding protein inhibits parasite growth during controlled human malaria infection. Sci Transl Med. 2023;15.

70. Di Angelantonio E, Thompson SG, Kaptoge S, Moore C, Walker M, Armitage J, et al. Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. Lancet. 2017;390(10110):2360-71.

71. UK Blood Services Joint Professional Advisory Group. Position Statement, Creutzfeldt-Jakob

Disease. vCJD Working Party of the Standing Advisory Committee on Transfusion

Transmitted Infections. 2015 [Available from: www.cjd.ed.ac.uk.

72. Roestenberg M, O'Hara GA, Duncan CJ, Epstein JE, Edwards NJ, Scholzen A, et al. Comparison of clinical and parasitological data from controlled human malaria infection trials. PLoS One. 2012;7(6):e38434.

73. Griffin P, Pasay C, Elliott S, Sekuloski S, Sikulu M, Hugo L, et al. Safety and Reproducibility of a Clinical Trial System Using Induced Blood Stage Plasmodium vivax Infection and Its Potential as a Model to Evaluate Malaria Transmission. PLoS neglected tropical diseases. 2016;10(12):e0005139.

74. Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, et al. Primaquine failure and cytochrome P-450 2D6 in Plasmodium vivax malaria. N Engl J Med. 2013;369(14):1381-2.

75. van Agtmael MA, Gupta V, van der Graaf CA, van Boxtel CJ. The effect of grapefruit juice on the time-dependent decline of artemether plasma levels in healthy subjects. Clin Pharmacol Ther. 1999;66(4):408-14.

76. CDC. Treatment of Malaria: Guidelines for Clinicians (United States) 2023 [Available from: https://www.cdc.gov/malaria/diagnosis_treatment/clinicians1.html#pregnant.

77. FDA. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in

Preventive Vaccine Clinical Trials [Internet]. 2007 [Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical.

23. APPENDIX A: Systematic Coronary Risk Evaluation (SCORE2)

The SCORE algorithm was created by the European Society of Cardiology. It was updated in 2021 to SCORE2. It considers risk factors such as gender, age, total cholesterol, systolic blood pressure and smoking status. The cell closest to an individual's age, cholesterol and systolic blood pressure values, indicates the 10-year total risk. For the UK population, chart in Figure 7 is used.

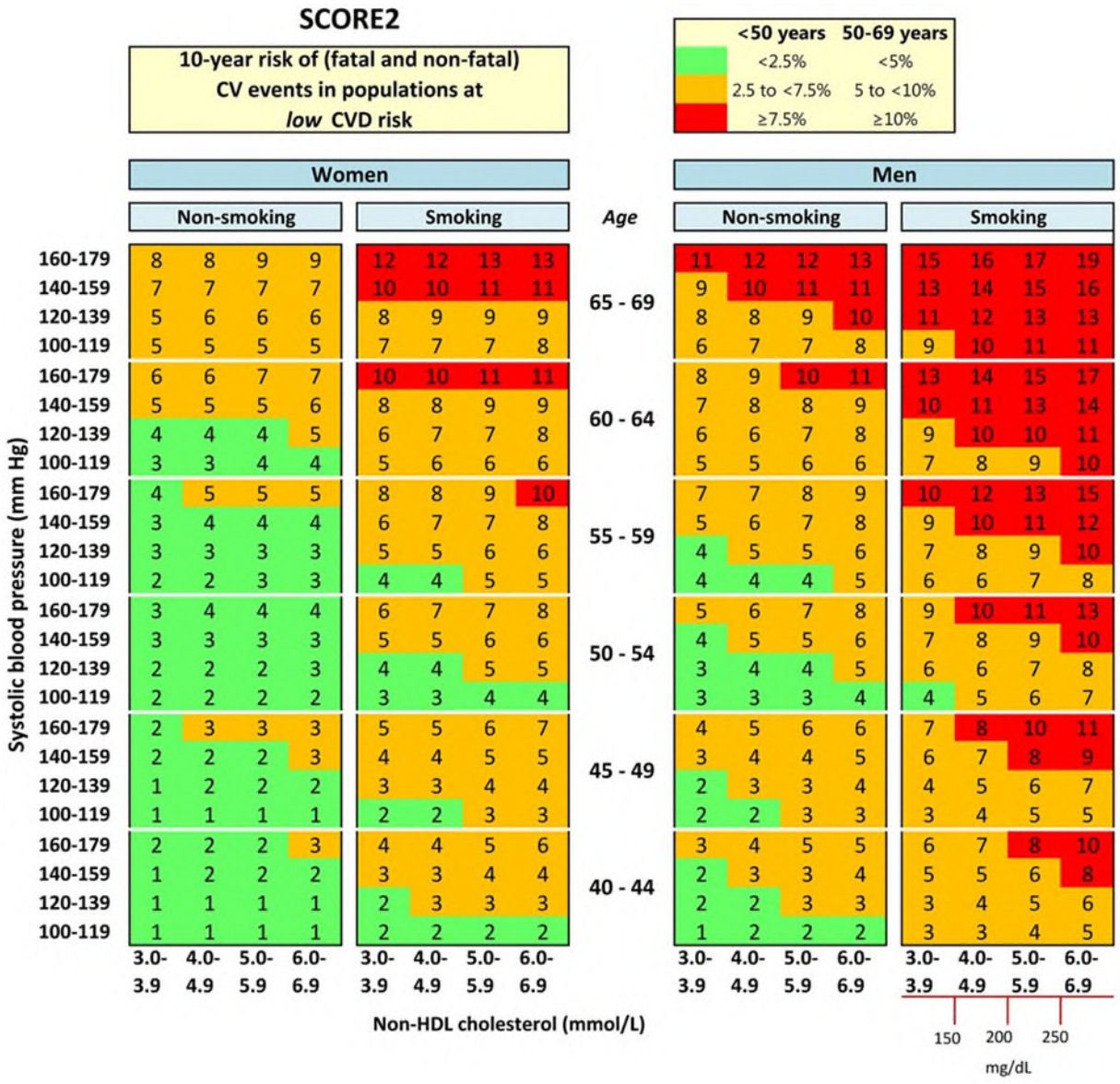


Figure 7: SCORE2 algoritm - European Low Risk Chart. 10 year risk of fatal CVD in low risk regions of Europe by gender, age, systolic blood pressure, total cholesterol and smoking status

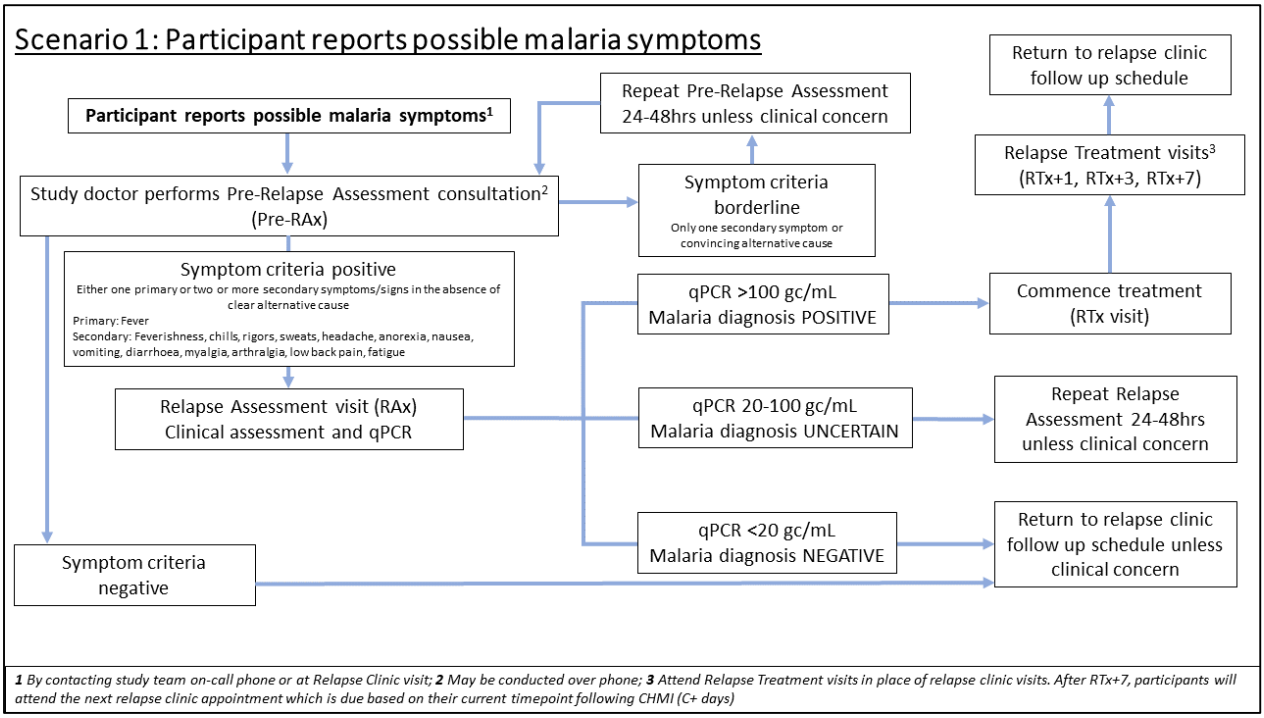
24. APPENDIX B: Grading of abnormal laboratory values

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with Investigator discretion for interpretation of results and the need for repeated or further tests. In general, volunteers will be not be included in the study if a repeat result at screening qualifies as a grade 1 (or higher) laboratory adverse event, according to the laboratory adverse event table (Table 15), and is deemed to be clinically significant.

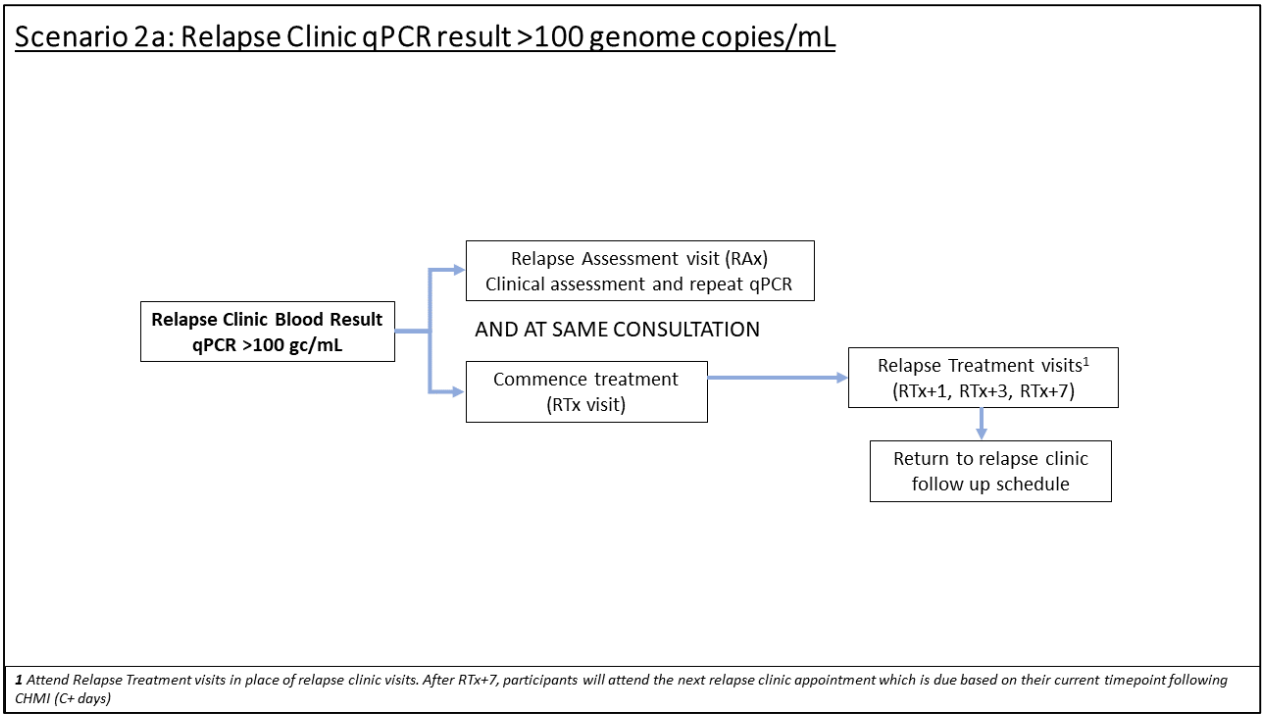
Table 15: Laboratory AE grading

	Grade 1 (mild)	**Grade 2 (moderate)**	**Grade 3 (severe)**
Haemoglobin: decrease from baseline value (g/L)	10 – 15	16 – 20	> 20
White cell count: Elevated (x 10^9/L)	11.10 – 15.00	15.01 – 20.00	> 20.00
White cell count: Depressed (x 10^9/L)	2.50 – 3.50	1.50 – 2.49	< 1.50
Neutrophil count (x 10^9/L)	1.50 – 1.69	1.00 – 1.49	< 1.00
Lymphocyte count (x 10^9/L)	0.75 – 0.89	0.50 – 0.74	< 0.50
Eosinophil count (x 10^9/L)	0.65 – 1.50	1.51 – 5.00	> 5.00
Platelet count (x 10^9/L)	125 – 149	100 – 124	< 100
Sodium: hyponatraemia (mmol/L)	132 – 134	130 – 131	< 130
Sodium: hypernatraemia (mmol/L)	146	147	> 147
Potassium: hypokalaemia (mmol/L)	3.3 – 3.4	3.1 – 3.2	< 3.1
Potassium: hyperkalaemia (mmol/L)	5.4 – 5.5	5.6 – 5.7	> 5.7
Urea (mmol/L)	8.2 – 9.3	9.4 – 11.0	> 11.0
Creatinine (µmol/L)	132 – 150	151 - 177	> 177
ALT (IU/L)	50 – 112	113 – 229	> 229
AST (IU/L)	46 – 105	106 – 213	> 213
Bilirubin, with increase in LFTs (µmol/L)	23.1 – 25	26 – 31	> 31
Bilirubin, with normal LFTs (µmol/L)	23.1 – 33	34 – 41	> 41
ALP (IU/L)	143 – 272	273 – 402	> 402
Albumin (g/L)	28 – 31	25 – 27	<25

25. APPENDIX C: Relapse assessment pathway and treatment algorithm

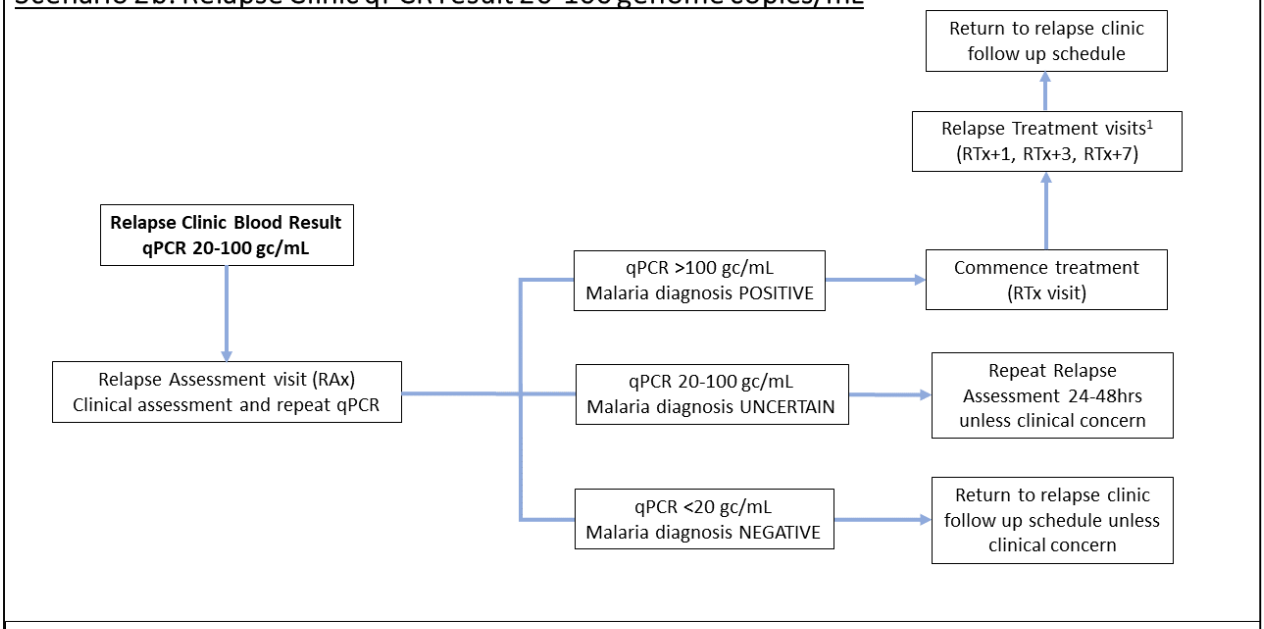


1 By contacting study team on-call phone or at Relapse Clinic visit; 2 May be conducted over phone; 3 Attend Relapse Treatment visits in place of relapse clinic visits. After RTx+7, participants will attend the next relapse clinic appointment which is due based on their current timepoint following CHMI (C+ days)



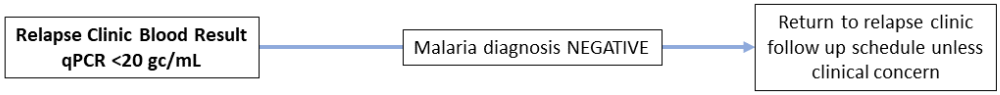
1 Attend Relapse Treatment visits in place of relapse clinic visits. After RTx+7, participants will attend the next relapse clinic appointment which is due based on their current timepoint following CHMI (C+ days)

Scenario 2b: Relapse Clinic qPCR result 20-100 genome copies/mL



1 *Attend Relapse Treatment visits in place of relapse clinic visits. After RTx+7, participants will attend the next relapse clinic appointment which is due based on their current timepoint following CHMI (C+ days)*

Scenario 2c: Relapse Clinic qPCR result <20 genome copies/mL



26. APPENDIX D: Be Part of Research Volunteer Service - HRA agreed wording to include in ethics submission

The purpose of the Be Part of Research Volunteer Service (BPORVS) is to allow members of the public to become volunteers by creating an account, specifying the areas of research that they are interested in and give consent to be contacted by the Be Part of Research team. Those who consent will receive information about BPORVS, in particular to alert them to specific BPORVS registered studies that they may be interested in, based on their volunteered details and study specific eligibility criteria, using an online self-registration service. The register is open to those that live in the UK, are over 18 and have an email address.

At the time of registration, volunteers are made aware that they are not signing up to take part in a specific health study when they join this register and that they will only be signposted to studies that have NIHR funding or are listed on the NIHR CRN Portfolio. If the volunteer is interested in the study there will be a link in the email to take them to the study team (e.g. website, pre-screener) where they will move into the study teams screening process and consenting process if they take part in the study.

The Be Part of Research Volunteer Service is funded by the Department of Health and Social Care and delivered by the National Institute for Health and Care Research (NIHR) in conjunction with Public Health Agency, Research & Development, Northern Ireland, NHS Scotland and Health and Care Research Wales. Further information on the Be Part of Research Volunteer Service is available here:

https://bepartofresearch.nihr.ac.uk/volunteer-service/researchers

