A Phase I/IIa clinical trial to assess the safety, immunogenicity and efficacy of the blood-stage *Plasmodium vivax* malaria vaccine candidate PvDBPII in Matrix M1 in healthy adults living in the UK

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Chief Investigator: Angela M. Minassian

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MODIFICATION HISTORY

Version	Date	Author(s)	Modifications
1.0	17th July 2019	Thomas Rawlinson Yrene Themistocleous Angela Minassian	Created
2.0	24th October 2019	Yrene Themistocleous	Revision of accepted methods of contraception for women of child bearing potential to include highly effective methods only Clarification of requirement for female participants using hormonal contraceptive methods to use an additional form of contraceptive until the next menstrual period, following initiation of treatment with artemether/lumefantrine
2.1	20th January 2020	Yrene Themistocleous	Minor corrections to schedule of visits table (clarifying total immunology bleed and volume of blood draw for haematology test at screening corrected to 4mL from 5mL)
3.0	14th February 2020	Thomas Rawlinson	Exclusion criteria relating to prior immunoglobulin exposure amended. Removal of thick blood film as diagnostic measure during CHMI Updates and corrections to schedule of visits and bleed volumes, including reduction of maximal blood draw Addition of timing windows for recording physical observations on day of challenge Clarifications to collection of adverse event data Potential for use of alternative antiemetic to cyclizine Change to group sizes (target range now specified) and removal of back up volunteers Clarification regarding timing of screening visits and for re-screening procedures for participants screened >90 days prior to enrolment CRP added as an exploratory measure Local safety monitor takes on role previously taken by local safety committee Additional wording in the protocol and PIS to clarify the repeat screening for blood-borne infection at day 96 post challenge

4.0	25th November 2020	Mimi Hou	Change of vaccination schedule for group 1 - delay of 3rd vaccination due to temporary trial halt Addition of Group 2, to be recruited if fewer than 6 participants complete the study in Group 1 Addition of serum bhCG to C+28/day of malaria diagnosis Clarification that G6PD, DARC and haemoglobinopathy screen are only done at NHS labs Extension of time window for study visits Changes to trial procedures to account for possibility of COVID-19 infection during CHMI. Addition of COVID-19 PCR swab test prior to challenge and on day of malaria diagnosis. Guidance on testing for COVID-19 if fever post vaccination and post challenge. Added option of using Malarone as first line anti-malaria treatment Correction of typographical errors Removal of measurement of T cell responses to PvDBPII by ELISpot from Secondary Immunological Outcome Measures Correction of error in calculation of total blood volumes in schedule of attendance table Addition of retrospective COVID-19 serology testing for exploratory analysis of effects of COVID serostatus on vaccine immunogenicity
5.0	19th January 2021	Mimi Hou	Clarification of exclusion criteria regarding concomitant vaccinations and addition of specific criteria relating to licensed COVID-19 vaccination Addition of section on administration of concomitant COVID-19 vaccination Extension of time window for 2nd and 3rd vaccinations Threshold at which new participants are recruited into Group 2 amended to if less than 8 participants complete study in Group 1 (previously less than 6 participants)
6.0	4th March 2021	Mimi Hou	Addition of exclusion criteria on concomitant COVID-19 vaccination around time of CHMI. Shortened time window of when COVID-19 vaccination can be given following malaria vaccination to aid scheduling of COVID-19 vaccinations. Updated section on Conduct of CHMI in the context of COVID-19 pandemic

			Removal of specific timeframe during which baseline observations are taken pre-challenge Correction of blood volume taken for HLA and total blood volumes
7.0	22nd July 2021	Mimi Hou	Addition of Group 3, comprising a subset of volunteers originally in Group 1, who consent to undergo a 4th vaccination and secondary CHMI. Addition of secondary objective to assess vaccine efficacy in Group 3. For Groups 2 and 3 - post-challenge follow-up visits changed to once a day until parasite count reaches >1000 genome copies/ml, then to continue twice a day visits until diagnosis. Latest day of treatment reduced to C+21. For Groups 2 and 3 - change of post malaria treatment visit from T+2 to T+3. Reduction in number of observed doses of antimalarial medication to two observed doses. For Group 2 – addition of immunology bleed at D42 Updated compensation table Correction of error in lumefantrine dose in section 8.5.5 Addition of reticulocyte count to FBC taken at C-2 visit. Removal of need to collect unused medications after completion of challenge. Correction under C+56 visit – no collection of any diaries occur at this timepoint. Correction of errors in protocol – only weight taken at C-2 visit. No CRPs are taken during post-challenge follow-up. Changed Senior Laboratory Investigator address. Addition of New Biochemstry Building as location for processing of research bloods.
8.0	21st June 2022	Mimi Hou	Samples will now be stored long term under University of Oxford's HTA license at Department of Biochemistry instead of Oxford Vaccine Centre Biobank.

A Phase I/IIa clinical trial to assess the safety, immunogenicity and efficacy of the blood-stage *Plasmodium vivax* malaria vaccine candidate PvDBPII in Matrix M1 in healthy adults living in the UK

Study Code: VAC079

Chief Investigator	Dr Angela M Minassian Centre for Clinical Vaccinology and Tropical Medicine, Jenner Institute, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE Email: angela.minassian@ndm.ox.ac.uk
Senior Laboratory Investigator	Prof Simon J Draper New Biochemistry Building, Department of Biochemistry, South Parks Road Oxford, OX1 3QU Email: simon.draper@bioch.ox.ac.uk
Trial sites	Centre for Clinical Vaccinology & Tropical Medicine Jenner Institute, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE PI at Site: Dr Angela M Minassian
Sponsoring Institution	University of Oxford Clinical Trials and Research Governance (CTRG) Research Services University of Oxford Joint Research Office Boundary Brook House

	Churchill Drive Headington OX3 7GB Tel: 01865 289885 Email: ctrg@admin.ox.ac.uk
External Monitor	Clinical Trials and Research Governance (CTRG) Research Services University of Oxford Joint Research Office Boundary Brook House Churchill Drive Headington OX3 7GB Tel: 01865 289885
Local Safety Monitor	Prof Brian Angus Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE Tel: 01865 220289 Email: brian.angus@ndm.ox.ac.uk

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, and members of the Independent Ethics Committee. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Dr Angela M. Minassian.

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 Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Dr Angela M. Minassian		23/06/2022
Chief Investigator	Investigator Signature	Date

Conflict of Interest

1. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no conflict of interest"

Dr Angela M. Minassian		23/06/2022
Chief Investigator	Investigator Signature	Date

Details:_____

2. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no conflict of interest"

Prof Simon J. Draper		23/06/2022
Senior Laboratory Investigator	Investigator Signature	Date

Details:_____

Table of Contents

1 SYNOPSIS

Title	A Phase I/IIa clinical trial to assess the safety, immunogenicity and efficacy of the blood-stage *Plasmodium vivax* malaria vaccine candidate PvDBPII in Matrix M1 in healthy adults living in the UK
Trial Centre	Clinical Centre for Vaccinology and Tropical Medicine, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE, UK
Trial Identifier	VAC079
Clinical Phase	Phase I/IIa
Design	Open label, first-in-human, Phase I/IIa, blood-stage *P. vivax* malaria vaccine trial
Population	Healthy adults aged 18 – 45 years
Sample Size	Total: 12-24 volunteers Up to 12 volunteers in Group 1 will receive three doses of the PvDBPII 50ug/Matrix M1 50ug candidate vaccine at 1, 2 and 12-18 months, prior to blood-stage CHMI 2-4 weeks after the third vaccination. If fewer than 8 volunteers complete the study in Group 1, then new volunteers will be recruited into Group 2, to make up a total of 10 to 12 volunteers who complete 3 vaccinations and CHMI between Groups 1 and 2. Group 2 volunteers will receive three doses of the PvDBPII 50ug/Matrix M1 50ug candidate vaccine at monthly intervals, prior to blood-stage CHMI 2-4 weeks after the third vaccination. Up to 6 volunteers who have completed 3 vaccinations and CHMI previously in Group 1, will be invited to undergo a fourth vaccination at 5 months after the third vaccination, followed by repeat CHMI 2-4 weeks after the fourth vaccination. These volunteers will comprise Group 3. All these volunteers will undergo blood stage CHMI with *Plasmodium vivax.* The de-identified data from volunteers in a parallel study (VAC069), who will undergo the same CHMI without prior vaccination, will be used as infectivity controls.
Follow-up duration	Participants in Group 1 will be followed for 9 months after the third vaccination, approximately 2 years in total from enrolment. Participants in Group 2 will be followed for 1 year from enrolment. Participants in Group 3 will be followed for 9 months after their final (fourth) vaccination, approximately up to 2.5 years in total from enrolment.

Planned trial period	2.5 years from the enrolment of the first volunteer.
Primary Objectives	To assess the safety of the PvDBPII-Matrix M1 vaccine in healthy volunteers To establish whether the PvDBPII-Matrix M1 vaccine can demonstrate a reduced parasite multiplication rate in vaccinated subjects compared to infectivity controls in a blood-stage controlled human malaria infection model
Secondary Objectives	To assess the humoral and cellular immunogenicity of the PvDBPII-Matrix M1 vaccine candidate. To assess immunological readouts for association with a reduced parasite multiplication rate (PMR) To assess the durability of any reduction in PMR in volunteers who undergo a fourth vaccination followed by re-challenge approximately 5 months after the primary challenge
Investigational Products	1. PvDBPII – a recombinant protein malaria vaccine candidate 2. Matrix-M1 – a saponin based vaccine adjuvant
Form	1. PvDBPII: liquid, stored between -70^{o}C and -90^{o}C 2. Matrix-M1: liquid, stored at +2^{o}C to +8^{o}C
Dose	1. PvDBPII - 50µg, administered in a three-dose schedule on days 0, 28 and 56 or 430. Fourth dose of 50µg given at day 580 for Group 3. 2. Matrix M1 - 50 µg, administered with each PvDBPII dose on days 0, 28 and 56 or 430. Fourth dose of 50µg given at day 580 for Group 3.
Route	Intramuscular injection in the deltoid region of the arm

2 ABBREVIATIONS

ACT	Artemisinin-based combination therapies
AE	Adverse event
AR	Adverse reaction
CBF	Clinical Bio-Manufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CHMI	Controlled human malaria infection
CI	Chief Investigator
CMV	Cytomegalovirus
CRF	Case Report Form or Clinical Research Facility
CTRG	Clinical Trials Research Governance
CYP2D6	Enzyme predicting Primaquine metabolism – Cytochrome P450 2D6
DARC	Duffy Antigen Receptor for Chemokines
DFA	Direct Feeding Assay
DSUR	Development Safety Update Report
EBV	Epstein-Barr Virus
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme linked immunospot assay
EPI	Expanded Programme of Immunisation
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HTLV	Human T cell Lymphotrophic virus
HLA	Human Leukocyte Antigen
ICH	International Conference on Harmonisation
IFN-γ	Interferon-gamma
IM	Intramuscular
IMP	Investigational Medicinal Product
LPS	Lipopolysaccharide
LSM	Local Safety Monitor
MFA	Membrane Feeding Assay
MHRA	Medicines and Healthcare products Regulatory Agency

MRC	Medical Research Council
μg	Microgram
NIHR	National Institute for Health Research
pIMD	potential Immune-Mediated Diseases
PCR	Polymerase Chain Reaction
PIS	Participant Information Sheet
QP	Qualified Person
qPCR	Quantitative polymerase chain reaction
RAS	Radiation-attenuated sporozoite
RUNMC	Radboud University Nijmegen Medical Center
REC	Research Ethics Commitee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UOXF	University of Oxford
USMMVP	United States Military Malaria Vaccine Program
VLP	Virus-like particle
WHO	World Health Organisation
WTCRF	Wellcome Trust Clinical Research Facility

3 BACKGROUND & RATIONALE

3.1 Impact of *P. vivax* malaria and the need for a vaccine

Plasmodium vivax (*P. vivax*) is one of the seven *Plasmodium* species known to cause human malaria and accounts for the most cases of non-*P. falciparum* malaria worldwide. Geographically, it is the most widespread human malaria parasite and approximately 2.5 billion people are at risk of contracting the infection in endemic areas [1]. It is estimated over one third of malaria cases outside of the African continent are due to *P. vivax* and that it accounts for 74% of malaria cases in the Americas, 37% in South-East Asia and 31% in the Eastern Mediterranean WHO regions. The burden is highest in Afghanistan, Ethiopia, India, Indonesia and Pakistan, which together account for 82% of *P. vivax* cases[2].

P. vivax malaria has long been considered, and termed, 'benign' malaria, but more recently large case series demonstrate that *P. vivax* infection is associated with significant morbidity and mortality. Complications reported include severe anaemia, respiratory and hepatic dysfunction, severe thrombocytopenia and coagulopathies, such as disseminated intravascular coagulation (DIC) [3]. *P. vivax* infection has also been associated with important sequelae for maternal and foetal health, correlating with increased rates of low birthweight, preterm delivery, and reduced foetal growth, as well as maternal and foetal deaths [4].

The socio-economic impact of this infection is not known, but in terms of overall global cost due to lost productivity this is estimated at US$ 1.4 to 4.0 billion per year [5]. Those affected by *P. vivax* malaria are typically poor with inadequate access to affordable healthcare and with little financial reserve, perpetuating the cycle of poverty [6].

Clinical cases not only result from primary infection but also relapses, which occur weeks to years after primary infection. Relapses are due to the ability of the *P. vivax* parasite to remain dormant in the liver in the hypnozoite stage, a lifecycle stage not seen with *P. falciparum* malaria [5]. Standard schizonticidal regimes are not effective against hypnozoites. Radical cure of dormant parasites requires therapy with the 8-aminoquinolone primaquine, currently the only licensed hypnozoitcidal anti-malarial, although tafenoquine, a new drug of the same class, given as a single dose, has recently been FDA-approved for adults in the United States. However, both 8-aminoquinolones carry a significant risk of severe haemolytic anaemia in individuals who are deficient in the glucose-6-phosphate dehydrogenase enzyme (G6PD), an inherited X-linked red blood cell enzyme disorder common in tropical and sub-tropical areas [7]. More recently, the importance of the cytochrome P450 enzyme, CYP2D6 in metabolism of primaquine to the active metabolite has been recognised [8, 9]. A common polymorphism in the *CYP2D6* gene for the cytochrome P450 enzyme results in poor conversion of primaquine to the active form, resulting in higher treatment failure rates. It is estimated that these two factors combined may make nearly 40% of the population at risk of *P. vivax* infection ineligible for primaquine therapy [7].

Control of *P. vivax* is also challenging, with re-emergence in areas where it has previously been eradicated. *P. vivax* is epidemiologically and biologically different to *P. falciparum* and control methods developed for *P. falciparum* are less efficient for *P. vivax* malaria [10-13]. Control efforts are complicated by multiple factors including difficulty detecting asymptomatic infection, resistance to antimalarials, a lack of understanding of parasite biology, early gametocytogenesis and relapses [14, 15]. Recent calls for control and 'eradication' of malaria worldwide [16] have focused attention on this neglected disease and the need for development of an effective *P. vivax* vaccine to be used alongside current control methods [17].

Consequently, the revised Malaria Vaccine Technology Roadmap to 2030 [17] now recognises the importance of *P. vivax* and calls for a vaccine to achieve 75% efficacy over two years – equally

weighted with *P. falciparum* in an era of renewed political will to move towards malaria elimination and eradication.

However, unlike *P. falciparum*, to date there has been relatively little research into this plasmodium species and one of the limiting factors has been the inability to culture *P. vivax* parasites *in vitro* over a prolonged period of time. More recently, research into *P. vivax* malaria has increased with candidate vaccines being developed and taken forward to clinical trial [18, 19]. Yet none of these vaccines have progressed past a Phase IIa trial, and options for assessing the immune responses to new candidates are extremely limited.

3.2 Lifecycle of the *Plasmodium vivax* malaria parasite

The lifecycle of *P. vivax* is complex, involving both the vector (the *Anopheles* mosquito) and the human host (Figure 1). Sporozoites are injected into the human host when the female *Anopheles* mosquito takes a blood meal. From here they migrate within minutes to the liver, where they invade hepatocytes and the schizont develops (liver-stage). The mature schizont ruptures after around 7 days, releasing merozoites into the blood stream, which invade reticulocytes preferentially (unlike *P. falciparum* which invades all erythrocytes). As well as developing schizonts in the liver, *P. vivax* forms hypnozoites, a dormant stage which develops into schizonts weeks to years later, rupturing when mature and releasing merozoites to cause blood-stage malaria. It is this stage of the lifecycle that is responsible for relapse.

Once merozoites have invaded reticulocytes they are termed rings, which again mature over 48 hours into mature schizonts which release merozoites. These merozoites go on to infect other reticulocytes, and the blood-stage cycle continues. A subset of rings develop into gametocytes, the sexual-stage of the malaria lifecycle, rather than forming schizonts. This occurs early in blood-stage infection with *P. vivax*, unlike *P. falciparum.* These are taken up by the mosquito in a blood meal. The sporogonic cycle takes place within the mosquito. The microgametocyte (male) exflagellates and enters the macrogametocyte (female) to form a zygote within the mosquito midgut. Zygotes become motile (ookinetes) and penetrate the midgut wall where they develop into oocysts. These rupture, release sporozoites which migrate to the mosquito salivary glands ready to be injected when the mosquito takes another blood meal [20].

The liver-stage of infection is asymptomatic. Symptoms, and subsequent complications, develop during the blood-stage.

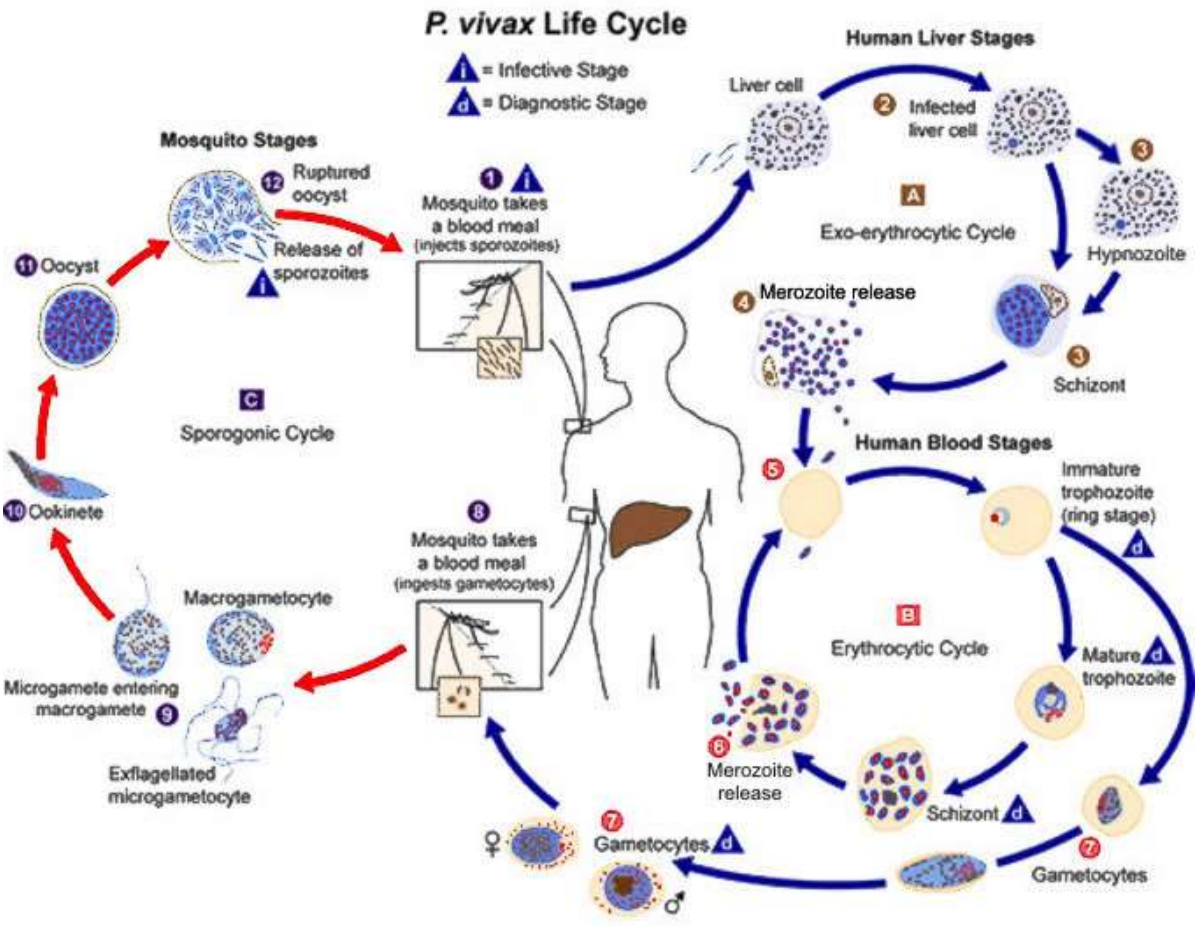

Figure 1: Lifecycle of *P. vivax* malaria

3.3 Challenges and directions in *P. vivax* vaccine development

The unique characteristics of *P. vivax* pose some specific challenges in studying its biology and designing strategies to control it. For example, the lack of a method for continuous culture of *P. vivax* blood-stages makes it difficult to perform *in vitro* growth inhibition assays to identify synergistic combinations of blood-stage antigens that can be targeted to inhibit *P. vivax* blood-stage growth with high efficiency. Several groups have recently succeeded in establishing short-term *P. vivax* culture for invasion assays using enriched reticulocytes from cord blood [21], but such methods are still dependent on access to fresh *P. vivax* isolates from malaria patients limiting the routine use of such assays to endemic regions. In the absence of a *P. vivax* blood-stage culture system, production of infected mosquitoes for sporozoite- or transmission-stage studies also requires access to *P. vivax* patients in endemic areas. In order to study hepatocyte invasion stages of *P. falciparum* as well as *P. vivax*, attempts have been made to establish *in vitro* liver models [22, 23]. In addition, a reliable humanised mouse model for *P. vivax* liver-stages is now available [24]. These approaches, together with the *P. cynomolgi* model in non-human primates will help to identify and test novel drugs and vaccines targeting hepatocyte stages [25]. Studies on expression of parasite proteins in hypnozoites will determine if these latent stages can also be targeted with novel drugs and vaccines.

Currently, the two most clinically advanced *P. vivax* vaccine candidates are both subunit vaccines based on *P. vivax* circumsporozoite protein (PvCSP), an analogue of the *P. falciparum* pre-

erythrocytic target, which have both have reached Phase IIa efficacy testing in mosquito-bite delivered, sporozoite *P. vivax* CHMI. The vivax malaria protein 1 (VMP001) vaccine, formulated in the GSK Adjuvant System $AS01_B$, was shown to be well tolerated and immunogenic in healthy US volunteers. However, following CHMI at 14 days after the third immunisation, the vaccine failed to induce any sterile protection, although a small but significant delay in parasitaemia was seen in 59% (16/27) volunteers compared to non-vaccinated controls. A Phase IIa trial assessing efficacy of another PvCSP based candidate (long synthetic peptide CS derivatives, formulated in Montanide ISA 51) in mosquito bite challenge is also now complete, with results currently awaited (Clinicaltrials.gov identifier NCT02083068).

To date, repeated exposure to radiation-attenuated sporozoites (RAS) delivered by mosquito bite has been the only "immunisation" strategy to demonstrate any protective efficacy in *P. vivax* CHMI. Protection following exposure to irradiated sporozoites was first demonstrated in 2 volunteers in the 1970's in Maryland but this result was not replicated in larger numbers until a recent study in Cali, Colombia [26, 27]. In the Cali study, twelve Duffy positive participants were exposed to seven "immunisations", receiving median of 434 infective bites (range 362–497) from irradiated, infected mosquitoes over 56 weeks. Volunteers were then treated with chloroquine and primaquine to clear any plasmodium infections that may have developed during the immunisation phase, before challenge at 8 weeks after the last RAS exposure. Immunised participants underwent CHMI alongside two controls, who were Duffy positive individuals exposed to non-irradiated, non-infected mosquitoes and seven controls who were Duffy negative individuals who had received the same immunisation regime. Sterile efficacy was demonstrated in 42% (5/12) of Duffy positive participants, interestingly, all of which were female (5/7 female participants vs.0/5 males) [27]. Given the apparent high dose exposure required to afford this level of protection, and the current lack of availability of cryopreserved, purified *P. vivax* sporozoites, this approach may be limited as a vaccination strategy but transcriptomic and anti-parasite antibody profiling may offer important insights into correlates of protection and new pre-erythrocytic vaccine targets.

Apart from the viral-vectored PvDBPII vaccine candidates [28], the only other *P. vivax* blood-stage vaccine currently in active clinical development is the recombinant protein PvDBPII vaccine. It targets the cysteine-rich, region II of PvDBP, which serves as the functional receptor-binding domain for the essential interaction for reticulocyte invasion with Duffy Antigen Receptor for Chemokines (DARC). A Phase I trial has demonstrated that the PvDBPII vaccine, formulated with the glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), is safe and induces functional strain-transcending antibodies, which inhibit the PvDBP-DARC interaction *in vitro* [29].

A single *P. vivax* transmission-blocking candidate, the recombinant protein Pvs25H, derived from the Pvs25 surface antigen of *P. vivax* ookinetes, has reached Phase I clinical testing. When administered in Alhydrogel, the vaccine produced promising transmission blocking activity as assessed in a direct membrane feeding assay (DMFA) in some volunteers, however, overall induced antibody titres were low [30]. To increase immunogencity, Pvs25H was tested in formulation with the stronger water-in-oil adjuvant, Montanide ISA51, but due to concerns regarding both local and systemic reactogenicity of this formulation, including erythema nodosum in two individuals, the trial was subsequently halted[31].

All vaccines currently in clinical development, and almost all those in pre-clinical stages, target individual stages of the parasite's lifecycle and are based on single antigens (Table 1 [18]). However, the development of an effective vaccine will likely require combination of multiple antigens that provide synergy to achieve high efficacy. For example, achieving high rates of blood-stage growth inhibition may require targeting a combination of key blood-stage antigens

involved in reticulocyte invasion. In addition, a combination of blood-stage antigens with liver-stage antigens may be needed to achieve high efficacy, and inclusion of antigens from sexual- and mosquito-stages may be needed to inhibit transmission. It is therefore necessary to initiate efforts to combine antigens both within and across developmental stages to achieve synergy and attain the goal of developing a vaccine for *P. vivax* malaria with high efficacy.

3.4 Rationale for a blood stage vaccine against *Plasmodium vivax* malaria

The feasibility of a blood-stage vaccine for *P. vivax* is supported by significant literature. Firstly, although the human immune system will attack almost any stage of the parasite life cycle in the human host, naturally-acquired immunity appears to primarily target blood-stage infections [32]. Naturally acquired immunity develops over time with repeated exposure, to both primary blood-stage infections and relapse infections in the blood, and individuals with acquired immunity may develop asymptomatic infection [5]. This is reflected in the epidemiology of disease; in high-transmission areas, most infections, and in particular severe infections, are seen in children whereas in low-transmission settings the risk of infection, and severe disease, is evenly distributed across age groups [33]. Naturally acquired immunity to *P. vivax* is complex and remains incompletely understood, but mechanisms of protection may include antibody mediated inhibition of invasion, enhancement of phagocytosis, cellular inhibition and neutralisation of malarial toxins produced during schizont rupture, as well as cytokine release [34].

Secondly, malaria occurs at the blood-stage of plasmodium infection. The potential advantage of a blood-stage vaccine would be that even if only partially effective, it would still confer some protection, with reduced disease severity, whilst allowing natural immunity to develop [35]. Conceptually, therefore, blood-stage malaria vaccines have significant advantages over vaccines targeting other life-cycle stages; if pre-erythrocytic vaccines fail to provide sterilising immunity, the individual would be vulnerable to severe blood-stage disease and mosquito-stage vaccines would offer no protection to the individual once infected. A strong case for malaria vaccine development is made by modelling studies which show the substantial benefit that would arise from deploying a vaccine that is only partially protective [36, 37]. These models, which typically assume a vaccine efficacy of 50%, and a protective half-life of 3 years, predict variable impacts on mortality, morbidity and parasite prevalence, depending upon intensity of transmission, deployment of other control methods, rate of immune decay and access to healthcare [36-38]. They all, however, reach general agreement that even with modest levels of efficacy, a vaccine would facilitate reductions in parasite prevalence if used in combination with other strategies. This role for a vaccine is very different to the 'magic bullet' envisaged in the past; a vaccine would form one arm of an integrated programme consisting of indoor residual spraying, long-lasting bed nets, draining of mosquito habitats and provision of artemisinin-based drugs. The effect of a hypothetical vaccine with ninety-five percent efficacy and a protective half-life of ten years has also been modelled [38], and in this scenario a single mass administration of such a vaccine could; eliminate malaria from low-transmission zones; eliminate malaria from moderate-transmission zones if used in combination with other methods; and reduce prevalence to below five percent in high-transmission zones.

The hypnozoite stage of *P. vivax* means that individuals who are infected are at risk of relapse of disease, and a blood-stage vaccine could, importantly, protect against this (as long as relapses occurred within the duration of protective blood-stage immunity provided by vaccination). Relapses in vivax malaria occur weeks to years after initial infection, and may arise 10-14 times after the primary infection in the absence of eradication therapy, occurring every 3-6 weeks (in tropical climates) [5].

Thirdly, the *P. vivax* Duffy-binding protein (PvDBP) is a very promising antigen for vaccine development (see section 3.5). *P. vivax* requires interaction between the Duffy-binding protein and the host Duffy antigen receptor for chemokines (DARC) in order for the parasite to invade reticulocytes during blood-stage infection. Populations who are Duffy antigen negative are protected from vivax malaria, as demonstrated by a geographical scarcity of the infections in the areas they inhabit (for example, much of sub-Saharan Africa). Furthermore, natural immunity has been found to be associated with anti-PvDBP responses [39-41].

Vaccine candidate	Development Phase	Lifecycle stage	Antigen	Delivery system
VMP001	Phase I/IIa END	Liver-stage	PvCSP	Rec. protein-AS01B
CSV-S,S	Pre-clinical	Liver-stage	PvCSP	HBsAg fusion-AS01B
PvCSP-LSP	Phase I END	Liver-stage	PvCSP	Synthetic peptides-Montanide ISA 720
ChAd63-PvTRAP/MVA-PvTRAP	Pre-clinical	Liver-stage	PvTRAP	Prime-boost, viral vectors
PvDBPII	Phase I	Blood-stage	PvDBP	Rec. protein-GLA-SE
PvDBPII-DEKnull	Pre-clinical	Blood-stage	PvDBP	Rec. protein
ChAd63-PvDBPII/MVA-PvDBPII	Phase I/IIa	Blood-stage	PvDBP	Prime boost, viral vectors
$PvMSP1_{19}$	Pre-clinical	Blood-stage	PvMSP1	Rec. protein-Montanide ISA720
PvAMA1	Pre-clinical	Blood-stage	PvAMA1	Rec. protein-adjuvant
Pvs25H	Phase Ia END	Transmission-stage	Pvs25	Rec. protein-Alhydrogel; Rec. protein-Montanide ISA 51
Pvs28	Pre-clinical	Transmission-stage	Pvs28	Rec. protein-adjuvant
Pvs25-IMX313	Pre-clinical	Transmission-stage	Pvs25	Rec. protein-adjuvant
AnAPN1	Pre-clinical	Mosquito midgut Ag	AnAPN1	Rec. protein-adjuvant

Table 1: *P. vivax* vaccine candidates under development: taken from Ref [18].

3.5 PvDBP as an Antigen

From the early 1920s until the advent of penicillin in the mid-1940s, induced malaria became the standard treatment of neurosyphilis. An observation made soon after the introduction of malariotherapy to the United States, was the refractoriness of most people of African origin to infection with *P.vivax*, as opposed to universal susceptibility of persons of other origins [42]. The basis for this resistance was not uncovered until the mid-1970s [43] , when it was demonstrated that Duffy blood group positivity was required for red blood cell invasion by *P. vivax*, and nearly all West Africans, and a majority of sub-tropical African populations, are Duffy-negative . Twenty years later the parasite ligand which interacts with the Duffy antigen receptor for chemokines (DARC), known as Duffy-binding protein (DBP), was identified.

Plasmodium merozoite invasion of erythrocytes is a complex process. The merozoite reorients after an initial interaction with the erythrocyte, so that its apical end faces the surface of the cell. A tight junction is formed between the merozoite and erythrocyte when the micronemes and rhoptries of the merozoite discharge their contents. *Plasmodium vivax* expresses homologues of the Py235 rhoptry proteins, referred to as PvRBP1a, PvRBP1b, PvRBP2a and PvRBP2b which bind to reticulocytes and not normocytes, presumably explaining the host-cell preference of *P. vivax* [44]. DBP, belongs to a family of microneme proteins that are functionally conserved across *Plasmodium* species and are thought to trigger release of rhotry proteins, which leads to the formation of a tight attachment between the parasite and target host cell. The erythrocyte membrane subsequently encases the merozoite, creating a vacuole around the invading parasite [45, 46].

Unlike *P. falciparum* which utilises multiple redundant invasion pathways for human erythrocyte invasion [44, 47], *P. vivax* requires interaction with the Duffy antigen receptor for chemokines (DARC). Further to population level evidence of the protection of individuals lacking the Duffy blood group antigen, as seen across much of sub-Saharan Africa, knockout of the orthologous *P. knowlesi* DBPα gene prevents invasion of Duffy-positive erythrocytes by this highly related parasite *in vitro* [45].

DBPs have been characterised for both *P. knowlesi* (PkDBP) [48] nd *P. vivax* (PvDBP) [49]. PvDBP has a molecular weight of approximately 140 kDa and a 330-amino acid cysteine-rich region within it, known as region II (PvDBPII), is predicted to be the domain responsible for binding to DARC [50].

It is proposed that PvDBPII dimers bind either one or two DARC ectodomains and distinct heterotrimeric and heterotetrameric architectures may form on binding [51, 52]. Structural studies using the highly related *P. knowlesi* DBPα protein have shown that region II of PvDBP is polymorphic, suggesting this region is under immune pressure. However, the receptor binding residues within PvDBPII are highly conserved, with polymorphic residues clustered at sites that are distal to the DARC binding residues [53]. In-keeping with this, induction of binding inhibitory antibodies against these residues does not commonly occur following natural exposure [54, 55]. Yet, when these antibodies have been identified, as in a prospective cohort study in Papua New Guinea, they have been associated with reduced risk of *P. vivax* infection, lower *P. vivax* parasite densities and strain-transcending protection [39].

Over recent years, the critical nature of the PvDBP-DARC interaction with the Duffy antigen receptor for merozoite invasion has been challenged, with various reports of *P. vivax* infection in Duffy negative individuals in the literature [56-60]. Currently, the mechanism of invasion of Duffy negative erythrocytes is incompletely understood, however, alternative pathways have been suggested through identification of novel erythrocyte invasion genes and PvDBP gene duplication

in clinical isolates by whole genome sequencing[61, 62]. PvDBP gene duplication has not been linked to cases of Duffy negative *P. vivax* infection, however it has been geographically correlated with regions also reporting Duffy negative infection, perhaps pointing to involvement of PvDBP, even in Duffy negative infection [63]. The further complexities of *P. vivax* erythrocyte invasion in both Duffy positive and negative individuals will continue to be elucidated, and may reveal important, potentially synergistic or additive antigenic targets. However, PvDBP-DARC interaction likely remains an essential, non-redundant pathway for merozoite invasion in the large majority of cases and PvDBP continues to be the current leading target for candidate *P. vivax* blood-stage vaccine development [34, 64], likely with a greater chance of success in comparison to the widely tested merozoite antigen targets of *P. falciparum,* which unlike *P. vivax* is not limited to invasion by a single pathway.

4 PvDBPII VACCINE DEVELOPMENT

4.1 PvDBPII in pre-clinical studies

Pre-clinically, antibodies blocking PvDBP-DARC interaction have been reproducibly induced by immunization using PvDBPII–based vaccines in small animal models, including in mice and rabbits, as well as in non-human primates [51, 52, 65, 66], and those raised against the *P.knowlesi* PkDBPα ortholog can block RBC invasion by this parasite *in vitro* [53]. Following intravenous challenge of New World *Aotus* monkeys with blood-stage *P. vivax*, longer pre-patent periods and lower parasitaemias were observed in animals immunised with PvDBP-RII in comparison to controls. Protection was conferred following immunisation with protein vaccine in Freund's adjuvant but not Montanide ISA 720 (although antibody levels induced by ISA 720 were surprisingly low in this study) [67]. Importantly, antibodies raised against recombinant Salvador I (SalI) strain PvDBPII in pre-clinical animal models can inhibit binding of heterologous polymorphic PvDBPII domains derived from diverse field isolates [52a]. Antibodies against PvDBPII have also been shown to inhibit reticulocyte invasion by diverse *P. vivax* field isolates [68, 69]. These observations suggest that antibodies produced following vaccination with PvDBPII should be able to block diverse strains of *P. vivax*.

4.2 PvDBPII in clinical studies

4.2.1 Clinical experience with a viral-vectored PvDBPII vaccine in a Phase Ia clinical trial

Safety and immunogenicity of the ChAd63 and MVA viral vectored vaccines targeting PvDBP_RII (Salvador I strain) were assessed in the VAC051 trial (Clinicaltrials.gov identifier: NCT01816113), an open-label dose-escalation Phase Ia study in 24 healthy UK adults in Oxford between 2013-2014 [28]. Vaccines were delivered by the intramuscular route in a ChAd63-MVA heterologous prime-boost regimen using an 8-week interval. ChAd63 PvDBP was administered to 24 healthy malaria-naïve volunteers at doses of 5×10^9 vp (n = 4) and 5×10^{10} vp (n = 20). 8 volunteers received ChAd63 PvDBP alone, with the first 4 receiving the low dose (5×10^9 vp), before 15 volunteers went on to heterologous prime-boost regimens, receiving MVA PvDBP at doses of either 1×10^8 pfu (n= 7) or 2×10^8 pfu (n=8).

ChAd63 PvDBP administered alone, and particularly when preceding MVA boost, induced strong PvDBP_RII –specific T cell responses, as measured by IFNγ ELISpot. IgG titres were marginal following the higher dose prime of ChAd63, but these were boosted to similarly high levels in all volunteers by both doses of MVA. PvDBP_RII –specific antibody-secreting cell and memory B cell responses were also observed following the MVA boost and the polyclonal immune serum inhibited the binding of multiple allelic variants of recombinant PvDBP_RII to N-terminal DARC. Data from this study confirms that the PvDBP antigen is immunogenic and induces strain-transcending antibodies, which are binding-inhibitory *in vitro*, when administered as a viral-vectored vaccine in healthy UK adults.

ChAd63-PvDBP and MVA-PvDBP were well tolerated and demonstrated a favourable safety profile in malaria-naive adults. There were no serious adverse events reported during the VAC051 trial, and the majority of vaccine-related adverse events were mild in nature.

The most frequently reported adverse event following intramuscular administration of ChAd63 PvDBP at either of the above doses was injection site pain, seen in the majority of individuals. Less frequently, erythema, swelling, warmth and itch were also reported. The overall median duration of local adverse events was between 1 and 4 days. The most common vaccine-related

systemic adverse events were fatigue, headache, nausea, myalgia and arthralgia, with a median duration of 1-2 days and being mostly mild in nature.

MVA PvDBP led to injection site pain in all fifteen volunteers. At a dose of 1 x 10^8 pfu, pain was mild in the majority of individuals but reported to be severe in 1/7 volunteers and 3/8 volunteers receiving the higher 2 x 10^8 pfu. Localised swelling was also seen, which was mild in the majority of individuals at both doses. Warmth, erythema and itch were seen less frequently and the majority of all local adverse events resolved within 4 days of vaccination. Systemically, the most commonly reported AEs were headache and feverishness, which were mild in the majority of participants. At the 2 x 10^8 pfu dose, there was increased frequency of moderate or severe local and systemic adverse events compared to the 1 x 10^8 pfu dose, however, no systemic adverse event was reported to be severe for more than 24 hours.

4.2.2 Clinical experience with PvDBPII with adjuvant GLA-SE in a Phase Ia clinical trial

Safety and immunogenicity of the PvDBPII (Salvador I strain) vaccine formulated in glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE) adjuvant was assessed in a single centre, phase I, randomized, controlled, dose-escalating, single-blind study in India in 2016 (CTRI/2016/09/007289) [29]. Three doses of the PvDBPII/GLA-SE vaccine were administered by the intramuscular route at 4 week intervals. The vaccine was administered to 27 healthy malaria-naïve volunteers at doses of 10 µg (n=9), 25 µg (n=9) and 50 µg (n=9). Another 9 volunteers received GeneVac-B (hepatitis B vaccine from Serum Institute of India) as a control arm of the study. The adjuvant used was glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), a synthetic Toll-like receptor 4 agonist (a proprietary adjuvant from Infectious Disease Research Institute, Seattle, USA).

Immunogenicity data demonstrated that all three doses of PvDBPII (10, 25 and 50 µg) elicited antigen-specific and receptor blocking serum antibody responses. The 50 µg PvDBPII/GLA-SE vaccine dose elicited the highest antibody response against PvDBPII and the most persistent binding-inhibitory antibodies against PvDBPII. Both Day 84 and Day 180 sera inhibited binding not only of the homologous PvDBPII Sal I allele but also of three other PvDBPII alleles (P, O and AH), which are commonly found in Papua New Guinea.

The vaccine candidate, PvDBPII/GLA-SE, which was tested in healthy Indian male adults, was safe and well tolerated. No significant immediate reactogenicity was observed within the first hour post immunization at all three dose levels (10, 25 and 50 µg PvDBPII formulated with GLA-SE [5 µg of GLA]). No SAE was observed and no subject withdrew or was withdrawn from the study on account of safety. A single mild local solicited AE (pain at injection site) after the first dose of 10 µg PvDBPII was reported. No systemic solicited AEs were observed. There were similar incidences and intensity of unsolicited AEs among the subjects who received PvDBPII/GLA-SE and those who received GeneVac-B (control hepatitis B vaccine). No clinically significant difference was observed in haematology, biochemistry and urinalysis parameters post vaccination. Vital signs showed no significant changes throughout the study. No abnormal findings were observed during the post-study physical examination. There was no difference in the number of subjects who had biological values outside the normal ranges for different haematological and biochemical parameters in the PvDBPII/GLA-SE and control vaccine group.

Description	**PvDBPII (10µg) N=9 n(%)**	**PvDBPII (25 µg) N=9 n(%)**	**PvDBPII (50 µg) N=9 n(%)**	**Hepatitis B (20 µg) N=9 n(%)**
Solicited Local Adverse Events				
Pain	1 (11.11)*	0.00 (0)	0.00 (0)	0.00 (0)
Swelling	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Erythema	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Induration	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Tenderness	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Solicited Systemic Adverse Events				
Fever	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Vomiting	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Diarrhoea	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Headache	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Fatigue	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Myalgia	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Note: N= Total number of subjects randomized, n= number of subjects assigned for individual a nalysis; %=(n/N)100;Exact Binomial proportion and 95% CIs are calculated using Clopper-Pearson Method used in SAS * Mild in severity				

Table 2; adverse events from the PvDBPII with adjuvant GLA-SE Phase Ia clinical trial [29]

4.3 Matrix M1

GLA-SE, the adjuvant used in the PvDBPII vaccine trial described above, has shown comparable immunogenicity to Alum in human clinical trials, and the antibody titres generated may not be sufficient to protect against malaria, where very high antibody titres are required. When the P. falciparum malaria VLP, R21, was tested in combination with Matrix M1 and compared head-to-head against R21 in combination with GSK's potent adjuvant AS01B, the immunogenicity in humans was the same (ClinicalTrials.gov identifier: NCT02572388), confirming the potency of Matrix-M1. We would naturally choose the best adjuvant for this proof-of-concept study, and so have elected to combine PvDBPRII with Matrix-M1

Matrix-M1 (MM) is a 40nm-sized complex containing the adjuvant-active saponin *Quillaja saponaria*, phospholipid and cholesterol. Quillaja saponins are triterpene glycoside substances derived from the tree *Quillaja saponaria*. The molecular weights of the different saponins range from 1800 - 2000 Da. In water, saponin in concentrations of 200-500 ppm exist as monomers; at higher concentrations they aggregate as micelles, with a molecular weight of approximately 100000 Da. Saponins are surface-active compounds with a variety of applications including in agriculture, feed, food and beverage, mining, and veterinary vaccines, and are currently being investigated in human vaccine clinical trials. In aqueous solution, saponins are excellent adjuvants and are used in commercial veterinary vaccines, e.g. vaccines against foot-and-mouth disease, bovine mastitis, feline leukemia and equine influenza. An HPLC-purified fraction of the same saponin, called QS21, is a component of the AS01 adjuvant used in the pre-erythrocytic vaccine RTS,S/AS01.

Matrix-M adjuvant, in 1 of 2 formulations (named Matrix-M1 or Matrix-M2), has been administered to 1669 individuals, of which 1132 have received Matrix-M1, in a total of 15 clinical trials in the US, Europe, and Australia. These trials include assessment of two pre-erythrocytic malaria candidate vaccines, ChAd63/MVA ME-TRAP, and the VLP R21, adjuvanted with Matrix-M1, within four Phase I and Phase I/II trials conducted in Oxford and Burkina Faso (ClinicalTrials.gov identifiers: NCT02572388, NCT02925403, NCT01669512, NCT02905019). Available safety data demonstrates that the Matrix-M adjuvant is well-tolerated and there have been no serious unexpected adverse reactions or adverse reactions of special interest reported to date.

The profile of adverse events following vaccination with PvDBPII-Matrix M1 may be partially predicted from previous studies assessing protein vaccines using the Matrix-M adjuvant. Local adverse events are likely to include injection site pain, erythema, swelling, itching and warmth. Expected systemic adverse events would include headache, fatigue, myalgia, arthralgia, malaise, feverishness, fever and nausea.

4.4 Concomitant COVID-19 vaccinations

Trial participants may become eligible for a licensed COVID-19 vaccine during the course of the study. During this trial, COVID-19 vaccines should not be received within 14 days before or 7 days after each malaria vaccination (compared to 30 days for any other vaccines) and the time window for the second and third vaccinations have been extended to -7/+14 days to aid scheduling of trial vaccinations around any COVID-19 vaccinations if required.

The antigen of COVID-19 vaccines differ significantly from the malaria antigen tested in this trial and the currently approved COVID-19 vaccines are different types of vaccines compared to the recombinant protein vaccine used in this trial. We therefore do not expect a significant impact on immunogenicity to the malaria vaccines if given outside of the time window specified.

In regards to safety assessment following malaria vaccinations, assessment of solicited adverse events will not be affected by COVID-19 vaccinations if they are given a minimum of 7 days after trial vaccinations. Assessment of unsolicited adverse events, in particular delayed events occurring after 2 weeks of any trial vaccinations may be more difficult to interpret. However assessment of causality will be based on timing and previous experience with the PvDBPII vaccine in the Phase Ia trial.

5 CONTROLLED HUMAN MALARIA INFECTION

5.1 Controlled human malaria infection studies

5.1.1 Microbial challenge studies of human volunteers

The deliberate infection of human volunteers with micro-organisms has contributed uniquely to our understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including *P. falciparum* malaria, influenza, cholera, typhoid and hepatitis [70]. A review by the UK Academy of Medical Sciences on microbial challenge studies recognised that such studies are desirable for providing proof of concept for prophylactic and therapeutic interventions and can significantly accelerate progress to Phase III studies [70, 71].

5.1.2 Controlled human malaria infection with *Plasmodium falciparum*

P. falciparum CHMI has now become established as a key tool to assess the efficacy of novel malaria vaccines and drugs. Following the development of protocols for the continuous culture of *P. falciparum* in 1976 [72] and the generation of mature *P. falciparum* gametocytes *in vitro* in 1981 [73], it became possible to produce laboratory-reared infectious mosquitoes, meaning that CHMI trials could be performed more routinely [74].

The first well-documented CHMI study with laboratory-reared infectious mosquitoes was carried out in 1986 at the US Walter Reed Army Institute of Research (WRAIR), the US Naval Medical Research Institute (NMRI) and the US National Institutes of Health (NIH) [75]. The following year, the efficacies of the first recombinant protein and synthetic peptide *P. falciparum* vaccines were reported for experimentally infected volunteers [76, 77].

There are now an increasing number of centres conducting CHMI worldwide, including in Africa, and between 1985 and 2009, a total of 1,343 volunteers were experimentally infected with *P. falciparum* [78, 79]. Established methods now include via direct intravenous injection of purified, aseptic, cryopreserved sporozoites, as well as blood-stage CHMI, induced by injection of cryopreserved parasitised erythrocytes [80]. These models, in addition to mosquito bite delivered sporozoite challenge have significantly contributed to the *P. falciparum* vaccine pipeline, including the most clinically advanced candidate RTS,S, which is currently being evaluated as part of a WHO pilot programme in Phase IV studies [81].

5.1.3 Controlled human malaria infection with *Plasmodium vivax*

In comparison to *P. falciparum*, there is much less experience with *P. vivax* in the modern CHMI era. A key bottleneck has been the inability to culture *P. vivax* long-term *in vitro*. Sporozoite challenge with *P. vivax* also carries the risk of relapse weeks to years after infection if hypnozoites are not cleared from the liver (there is no dormant liver form in the *P. falciparum* lifecycle). However, there is an extensive history of deliberate infection with *P. vivax*; most notably in malariotherapy, which was carried out for the treatment of neurosyphilis almost a century ago. The Austrian psychiatrist Julius Wagner-Jauregg later received a Nobel Prize for his work with this treatment [82] and the practice was widely adopted as the only effective treatment available at the time. Malariotherapy provided a wealth of information about *P. vivax* infection, which has been reviewed previously [83]. Deliberate infection with *P. vivax* was also conducted in the USA from the 1940s to 1970s in prisoners involved in the Malaria Research Project at the Illinois State Penitentiary. The studies mainly examined compounds for their potential use as antimalarials, but also assessed the ability to immunise following exposure to *P. vivax* infected irradiated mosquitoes, followed by challenge with non-radiated infected *Anopheles* [84, 85]. Similar studies were also carried out at the United States Penitentiary, Atlanta

and Maryland USA. 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 [43]. These experiments demonstrated that CHMI with *P. vivax*, as well as *P. falciparum,* could be successfully carried out, but the studies were very small.

5.1.4 *P. vivax* sporozoite challenge in the modern era

More recently, over the last few years, a handful of *P. vivax* challenge studies have been reported. The majority of these studies have been mosquito-bite delivered sporozoite challenge trials, conducted in Cali, Colombia, the Walter Reed Army Institute of Research (WRAIR), Maryland, USA [86] [70] [88] and Oxford (VAC068 study, ClinicalTrials.gov Identifier: NCT03377296, unpublished).

A total of four sporozoite challenge studies have been conducted in Cali. 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. Authors speculated that the volunteer who did not develop malaria had surreptitiously taken anti-malarial medication but this was never confirmed [86]. There were no serious adverse events (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 [87]. 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) [88]. Symptoms were significantly worse among the malaria-naïve subjects but there were no SAEs.

The most recent CHMI trial from the Cali group assessed "immunisation" through repeated exposure to radiation-attenuated sporozoites, delivered by mosquito bite. 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 [27].

The first *P. vivax* sporozoite challenge in Europe was recently conducted in April 2018, in Oxford within the VAC068 study (ClinicalTrials.gov Identifier: NCT03377296). Two malaria-naïve adult volunteers were exposed to five infectious bites (bites by mosquitos with >10 sporozoites as detected on microscopic examination of salivary glands post-feeding) 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. Both volunteers were successfully infected, with parasitic DNA being detectable from day 9 after challenge by qPCR in both subjects. Symptoms consistent with malaria infection, including fatigue and subjective feverishness, chills, headache, loss of appetite, nausea and malaise, were reported by one volunteer from day 11 after challenge and on day 14 by the second volunteer. Fever was detected in both subjects on day 14. Thick film microscopy was positive (≥2 morphologically normal asexual parasites seen in 200 high-power (1000x) fields) in the asymptomatic volunteer on the evening of day 13 and at day 14 in the more

symptomatic volunteer, leading to diagnosis on day 14 in both volunteers. A 250mL blood donation was made by each volunteer, immediately prior to initiation of anti-malarial treatment, without complication, for cryopreservation and future use as a parasitised blood inoculum for blood-stage challenge. After 48 hours' anti-malarial treatment, both thick film microscopy and qPCR were negative (two consecutive substantially reduced results) and both volunteers completed the clinical follow-up period, including a two-week course of primaquine for radical cure of hypnozoites, until 90 days post-challenge, without safety concerns. To-date (more than 11 months post completion of primaquine therapy) there has been nothing to suggest relapse in either volunteer (email questionnaire follow-up is being continued for 5 years).

5.1.5 *P. vivax* challenge for assessment of vaccine efficacy

In only two of the *P. vivax* CHMI studies conducted to date has efficacy of immunisation been assessed, with both candidate vaccines targeting the pre-erythrocytic stage and utilising the mosquito-bite delivered sporozoite challenge model (Table 2 – taken from Payne *et al*., Trends in Parasitology, 2017 [89]). Only one of these studies has been published to date; the VMP001/AS01B vaccine was tested in healthy malaria-naïve adults at the WRAIR in the USA [90]. VMP001 is a soluble recombinant protein vaccine [91], encoding the *P. vivax* circumsporozoite protein (PvCSP) administered with the AS01B adjuvant (GlaxoSmithKline). The vaccine was administered to 30 volunteers in three cohorts (10 in each) at doses of 15 µg, 30 µg and 60 µg, given three times at a 4 week interval between the first and second dose; the third dose was given 8 (15 µg cohort), 6 (30 µg cohort) or 4 (60 µg cohort) weeks after the second. Twenty-nine volunteers completed the vaccination phase, with twenty-seven proceeding to sporozoite CHMI, delivered by mosquito bite, two weeks after final vaccination, alongside 6 malaria-naïve controls. Vaccine protective efficacy was 0%; all volunteers had developed thick blood film-detectable parasitaemia by day 13. The median pre-patent period for all immunised participants was 11.9 days versus 10.7 days for infectivity controls. The challenge was well tolerated with no untoward reactogenicity following mosquito bites and no untoward SAEs in challenge and treatment phases. Participants were treated with standard chloroquine and primaquine therapy with rapid clearance of infection. However, two volunteers went on to have multiple relapses. One participant experienced two relapses (at weeks 8 and 18 after CHMI), while the other experienced three (at weeks 11, 20 and 48 after CHMI) [9]. By study completion the participants had been followed up for 5 years, and had not had any further relapses [90]. Exploratory genotyping for the cytochrome P450 (CYP) allele CYP2D6 was undertaken in 25 of the 33 volunteers. The volunteers with relapses 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 [9]. Results from another *P. vivax* CSP vaccine candidate, CS long synthetic peptides formulated in Montanide ISA 51, assessed in a mosquito-bite challenge study are currently awaited (Clinicaltrials.gov identifier NCT02083068).

Trial Site	Number of volunteers	Pre-patent period (days)[a]	Number of infected mosquitoes OR inoculum	Number of volunteers with patent parasitaemia	References
Sporozoite (mosquito-bite) CHMI studies					
Cali, Columbia	18	9 – 13	2 - 10	17/18	[86]
Cali, Columbia	17 Duffy positive 5 Duffy negative	9 – 16	2 - 4	17/17 (Duffy positive) 0/5 (Duffy negative)	[87]
Cali, Columbia	7 malaria-naïve 9 semi-immune	11 – 13	2 - 4	16/16	[88]
WRAIR, USA	27 vaccinees 6 infectivity controls	10 – 13 10 – 11	5		[90]
Cali, Columbia	12 Duffy positive vaccinees 2 Duffy positive controls 5 Duffy negative controls	12 – 13	2 - 4	7/12 vaccinees 2/2 Duffy positive controls 0/5 Duffy negative controls	[27]
Blood-stage CHMI studies					
QIMRB, Australia	2	8-9	13,000 genome equivalents	2/2	[92]
QIMRB, Australia	6	8-9	31,786 (± 11,947) genome equivalents (= 15 ± 5 viable *P. vivax* parasites)	6/6	[93]

Table 3: Overview of Published *Plasmodium vivax* CHMI Studies. [a]The pre-patent period refers to the period before malaria diagnosis: by blood film (sporozoite) or qPCR (blood-stage).

5.1.6 Modern blood-stage controlled human *P. vivax* infection

To date, there have been four studies of blood-stage *P. vivax* CHMI, of which two are published, all conducted at the Queensland Institute of Medical Research (QIMR), Brisbane Australia. Both published studies used a cryopreserved source of parasitised erythrocytes obtained from a returned traveller with blood group A, Rhesus negative, who was infected with *P. vivax* during a trip to the Solomon Islands [92, 93]. The first blood-stage *P. vivax* CHMI in Europe took place in January 2019, in Oxford (VAC069) – see section 5.2.7 for a full description.

In the first blood-stage CHMI at QIMR, Brisbane, two volunteers were inoculated with 270µL of reconstituted parasitised red cells, containing approximately 13,000 genome equivalents. Both subjects were successfully infected, with PCR becoming positive on day 8 or 9. Clinical symptoms consistent with malaria infection were present from day 11 in one volunteer, who reported myalgia, headache and malaise and day 14 in the other, with both experiencing chills and sweats at day 14. Treatment with artemether/lumefantrine was started at day 14 and volunteers were admitted for ongoing monitoring. Both volunteers experienced worsening of symptoms over the initial 12 hours post-treatment, including nausea and vomiting, headache, sweats and rigors in one volunteer. Pyrexia above 39^{o}C was detected in both volunteers. After 24 hours of anti-malarials, all symptoms had resolved, although non-tender clinically palpable mild splenomegaly was detected in one subject at day 15. By day 28, splenomegaly had resolved fully and no other adverse events were reported by either subject in the period since discharge. Whole genome sequencing was performed, demonstrating a genetic sequence most closely related to Cambodia strains, of the publically available strains. Gametocytaemia was measured by quantitative reverse-transcriptase PCR for the *pvs25* transcript, a mature gametocyte marker. *Pvs25* transcripts were detectable from day 11 or 12 and peaked at day 14 in both volunteers, coinciding with peak quantitative parasite PCR, prior to the initiation of anti-malarial treatment [92].

A second study was conducted to demonstrate reproducibility of the blood-stage controlled infection method and assess transmissibility of infection to *Anopheles stephensi* mosquitoes. Six volunteers were infected after receiving an inoculum containing approximately 31,786 (+/-11947) genome equivalents, estimated by investigators using linear regression growth modelling to equate to 15+/-5 viable parasites. Evolution of clinical symptoms was similar to the first study, with volunteers developing symptoms in keeping with parasite infection on days 11-13 (mean 12.2). There were no serious adverse events during the course of either study, but for 4 of 6 volunteers, liver enzymes became significantly elevated, above 5 times the upper limit of normal. Mild bilirubinaemia was also detected in three subjects, however, increases were transient and did not exceed 2 times the upper limit of normal. No clinical manifestations were associated with biochemical abnormalities. No other cause for this deranged liver function was found and liver ultrasound was normal but abnormalities subsequently resolved spontaneously without sequelae [93]. Transmissibility of infection was assessed by both direct feeding assay (DFA) and membrane feeding assay (MFA), carried out 3 days prior to the anticipated peak parasitaemia and initiation of treatment on day 14, as predicted from the first study. A total of 16 DFAs and 32 MFAs were performed, with rates of successful feeding above 90% across the two methods, without significant difference between the two. Overall, 1801 mosquitoes were dissected for examination of oocysts 7-9 days after feeding, of which 1.8% (n=32) were demonstrated to be infected, although the rate of infectivity was five times higher for DFA when compared to MFA. Other atypical ovoid structures were also detected by investigators on the mosquito midgut, which could not be identified by PCR as either microsporidia or fungal pathogens, known to infect mosquitoes. For standardisation of the method, if used for drug or vaccine efficacy

evaluation, other methods for confirmation of oocysts will be required. The reason for low infectivity could not be determined, however, a possible incompatibility of the Solomon Island derived parasite strain and the laboratory-reared *A. stephensi* colony, which had originally been sourced from India, was cited by the authors as a potential contributory factor. While small, these studies demonstrate the safety, feasibility and reproducibility of a *P. vivax* blood-stage challenge model and show the potential for use as a transmission model for evaluation of transmission-blocking vaccine candidates, although the methodology is yet to be optimised.

5.1.7 Rationale for blood-stage controlled human malaria infection

Mosquito-bite CHMI most closely models natural infection and the robustness and reproducibility of the *P. vivax* mosquito-bite delivered sporozoite challenge model has recently been demonstrated by Herrera *et al.* in South America [86, 87]. More recently, the safety and feasibility of a sporozoite *P. vivax* challenge, was demonstrated for the first time in Europe in April 2018 within the ongoing VAC068 study in Oxford (ClinicalTrials.gov Identifier: NCT03377296). Since cryopreserved *P. vivax* sporozoites are yet to be developed for human trials, mosquito-bite delivered CHMI will remain the mainstay for CHMI in assessment of pre-erythrocytic vaccines and drugs. However, for vaccines and anti-malarials targeting the erythrocytic stage, the blood-stage offers a number of advantages over sporozoite CHMI.

Infection of mosquitoes requires a ready supply of blood containing gametocytes, from an infected patient. Without the possibility of long-term *P. vivax* ex-vivo culture, supply is limited to endemic areas. For CHMI studies conducted in non-endemic zones, this necessitates infection and importation of mosquitos from an appropriate area, with suitable entomological facilities and expertise, which may be logistically demanding, particularly in cases of vaccine efficacy studies, where timing of challenge may be critical.

Blood stage CHMI also permits both better characterization and standardization of the parasite inoculum. Parasite density of the cryopreserved isolates can be established, increasing homogeneity of initial parasite inoculum density, when compared to sporozoites delivered by mosquito bite, and allowing the possibility of controlled variations of the administered parasite load. Full genome sequencing, including screening for mutations known to be associated with drug resistance can also be performed prior to challenge. In addition, safety data can be accrued through multiple uses of the same inoculum. Together, standardisation of strain and density permit for better comparison between candidate vaccines, when evaluated through challenge using the same inoculum source.

Since parasitic load introduced via intravenous injection is smaller than that released following schizogony at the liver-stage, as in natural infection and sporozoite challenge, there is a longer period of blood-stage multiplication. This provides an extended period for assessment of parasite multiplication rate (PMR), before the development of clinical symptoms or diagnostic criteria might be reached. In addition, comparison of PMR between infectivity controls from blood-stage CHMI performed in Oxford within the VAC054 trial, assessing efficacy of the *P. falciparum* apical membrane antigen 1 (AMA1) vaccine candidate, FMP2.1/AS01, and historic controls challenged by mosquito-bite, illustrated that although mean PMR was comparable, blood-stage challenge produced a much narrower range of PMR (smaller standard deviation with tight 95% confidence intervals). This provided greater power to detect partial vaccine efficacy, making blood-stage challenge a more robust tool for evaluation of candidate blood-stage vaccines [94]. This was indeed borne out in the recent Phase IIa efficacy study of the candidate vaccine RH5.1/AS01B, where the blood-stage challenge model was able to reliably detect a 17% reduction in the PMR between vaccinees and infectivity controls, with a high degree of confidence (P<0.02) (VAC063, ClinicalTrials.gov Identifier: NCT02927145).

Significantly, in addition to advantages seen with *P. falciparum* blood-stage CHMI, *P. vivax* blood-stage challenge provides a mode of infection which by-passes the liver stage. This eliminates the possibility of hypnozoite formation and therefore, risk of relapse. Equally, there is no requirement for CYP2D6 phenotype screening and primaquine therapy. A *P. vivax* blood-stage challenge is therefore less restrictive and confers important safety benefits over sporozoite challenge.

5.2 Cryopreserved *Plasmodium vivax* blood inoculum

5.2.1 Source and preparation of the cryopreserved inoculum

The cryopreserved parasitized red blood cells that will be used were produced by Dr Angela Minassian, Prof Simon Draper and colleagues at the University of Oxford in April 2018, as part of the VAC068 study (ClinicalTrials.gov Identifier: NCT03377296), approved by the South Central - Oxford A Research Ethics Committee.

5.2.2 Collection of *P. vivax* infected erythrocytes

Parasitised erythrocytes were collected from two *P. vivax*-infected volunteers (otherwise healthy), who donated blood following sporozoite *P. vivax* CHMI, delivered by mosquito bite. Volunteers were infected via five infectious bites (defined as bites by mosquitoes with >10 sporozoites as detected on microscopic examination of salivary glands post-feeding) from infected laboratory-reared *Anopheles diru*s mosquitos, under controlled conditions. Infected mosquitos were provided by, and imported from, 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 mosquitos, 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 PCR analysis confirmed *P. vivax* mono-infection. Molecular speciation by PCR (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.

Both UK volunteers ("Donors") were followed 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 PCR. Clinical symptoms consistent with malarial infection were reported from day 11.5 post-challenge in Donor 1 and from day 14 in Donor 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 volunteer 1 and at day 13.5 in volunteer 2, corresponding to 31,010 and 16,717 genome copies/mL by PCR, respectively. Blood donation was performed in each volunteer immediately prior to initiation of treatment on the morning or afternoon 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 will continue for 5 years in order to monitor for relapse.

5.2.3 Cryopreservation and storage of blood bank

After collection, blood was maintained at 37°C and transported immediately to the Jenner Institute Laboratory, University of Oxford. All laboratory processing was conducted under GMP-like conditions, under QP and 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 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 RBC-Glycerolyte mixture was aliquoted into 1.5mL cryovials and frozen at -80°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 are stored on behalf of the University of Oxford. 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 SOPs.

5.2.4 Infectivity and characterisation of parasite stock

Confirmation of parasite density within the blood collected for cryopreservation was performed via microscopy and quantitative PCR 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. Quantitative PCR 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 PCR 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, quantitative PCR 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, we intend to proceed with further testing of cryopreserved samples from Donor 1, and will use this as the challenge inoculum in this and future studies. Further testing will include whole genome sequencing to further characterize the parasitic strain, with particular scrutiny for SNVs present in the PvDBP gene and the five putative drug resistant genes *pvdhfr*, *pvdhps*, *pvcrt*, *pvmdr* and *pvmrp*.

5.2.5 Testing of source patient and blood donors for blood-borne and mosquito-borne infections

Initial testing for blood-borne infections and mosquito-borne disease was carried out on blood samples from the original source patient in Thailand, taken at the time of *P. vivax* diagnosis. Since *Anopheles* is a known vector of *Wucherichia bancrofti*, the main causative agent of lymphatic filariasis, blood was screened for filarial disease via rapid diagnostic test for IgG_4

antibodies to the *W. bancrofti* Wb123 antigen (SD BIOLINE Lymphatic Filariasis IgG_4) at the Thai field site. In addition, although there is no evidence that blood-borne infections can be transmitted by mosquito bite, for maximal assurance of safety, serology for HIV-1 and HIV-2, HTLV, Hepatitis B and C and syphilis were performed on a serum sample from the Thai source patient, at Oxford University Hospitals NHS Trust. Due to anecdotal reports of Japanese B encephalitis and Chikungunya in *Anopheles* species, blood from the source patient was also screened for both arboviral infections by PCR, as performed by the WRAIR unit, who used the same mosquito source for a CHMI trial that assessed the efficacy of the VMP001/AS01 *P. vivax* circumsporozoite recombinant protein vaccine[90]. As a further precautionary measure, although *Anopheles* species are not known to be vectors of Dengue, Zika or West Nile virus, on the independent advice of clinical Tropical Medicine specialist, Prof. Nicholas Day, Professor of Tropical Medicine and Director of the Mahidol Oxford Tropical Medicine Research Unit in Thailand, PCR for these infections was performed on the source patient's blood. In summary, molecular analysis (PCR) for Chikungunya, Japanese B encephalitis, West Nile, Dengue and Zika viruses was performed on whole blood at the Rare Imported Pathogens Laboratory (RIPL) in the UK. All source patient infection screen tests were negative.

Both UK volunteers ("donors") were subjected to screening for blood-borne infections, in line with the Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee guidelines https://www.transfusionguidelines.org/red-book/chapter-9-microbiology-tests-for-donors-and-donations-general-specifications-for-laboratory-test-procedures/9-1-general-requirements. Testing comprised serological tests for HIV-1 and HIV-2, hepatitis B and C, syphilis (anti-treponemal Ab) and HTLV-1 and HTLV-2 at screening, and nucleic acid amplification tests for HIV-1 and HIV-2 and hepatitis B and C, as well as repeat serological tests for HTLV-1 and HTLV-2 and syphilis 7 days before sporozoite challenge. Neither volunteer had travelled to any area which represented a risk of exposure to any other form of blood-borne infection. All tests were negative. Volunteers also underwent repeat serological testing for HIV, hepatitis B and C, syphilis, HITLV-1 and HTLV-2, 90 days after challenge was undertaken, to ensure that no seroconversion from a recently-acquired infection (that may have been undetectable around the time of challenge) had occurred since the challenge period. All tests remained negative.

In addition, volunteers were screened for Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) prior to enrolment. Both volunteers were IgG seropositive for both infections. However, leukodepletion has been shown to be effective in preventing transmission of both EBV and CMV [95] and PCR testing for both infections on the inoculum was negative, therefore, EBV-CMV concordant serostatus would not be considered a requirement for recipients of this donated blood in this study. This is in line with conduct of previous *P. falciparum* CHMI studies at Oxford, which have utilised a cryopreserved source of infected erythrocytes from a donor who was seropositive for both EBV and CMV. Initial studies using this inoculum had initially required that participants in CHMI trials be seropositive for both infections. However, following the development of PCR assays for both infections, molecular analysis of the inoculum was negative for both EBV and CMV. Following a risk-assessment, QIMR, Australia, subsequently removed seropositivity to these viruses as a criterion for participation as a recipient of the inoculum. In a previous *P. falciparum* challenge study (VAC054) at Oxford, 63% of volunteers were seronegative for CMV and 11% were seronegative for EBV pre-CHMI, and no cases of seroconversion were recorded post-CHMI [94]. Volunteers will however be informed of the theoretical risk of transmission of infection and this will be included within the consent form. Serostatus will be tested prior to CHMI at screening for volunteers as part of this protocol, and will be re-tested post-challenge. Serum samples post-CHMI will also be stored, for serostatus analysis of any infection, should this be required or requested by the Chief Investigator.

5.2.6 Sterility and screening for blood-borne infections of cryopreserved blood bank

Blood collected for cryopreservation was tested for bacterial contamination using validated GMP and GLP laboratory techniques. These included tests of sterility by direct inoculation, mycoplasma specific culture and detection and quantification of bacterial endotoxin by kinetic chromogenic limulus amoebocyte lysate assay. All of these assays were performed by SGS Vitrology, Glasgow, UK, using assays compliant with harmonised European Pharmacopeia and United States Pharmacopeia standards.

A further screen for blood-borne infections was conducted on the plasma, derived directly from the blood bank (separated from the red cells prior to freezing down), in line with testing procedures performed by the NHS Blood Transfusion service. DNA PCR for HIV-1, hepatitis B, hepatitis C, EBV, CMV, serology for HIV-2 and serology for HTLV-1, HTLV-2, and *Treponema pallidum* (RPR) was performed on thawed plasma samples at University Hospitals Birmingham NHS Foundation Trust laboratory. All tests were negative.

5.2.7 Determination of safety, feasibility and dose of cryopreserved *P. vivax* inoculum

Safety and feasibility of blood-stage *P. vivax* challenge, using the same bank of cryopreserved *P. vivax* infected erythrocytes as will be used in this trial, was assessed for the first time by CHMI in the VAC069 study in January 2019 (ClinicalTrials.gov identifier: NCT03797989). This proof-of-concept assessed feasibility of infection at three different doses of inoculum, by injection at three dilutions, with a view to identifying the lowest concentration producing a reliable infection within a practicable timeframe.

Six healthy, malaria-naïve adults aged between 18 and 50 years were recruited into three groups, each comprising two volunteers. Two volunteers receive a whole vial's worth of infected erythrocytes, two volunteers received one fifth of the dose of parasitised erythrocytes, at a 1:5 dilution, and the final two volunteers were inoculated with one twentieth of the dose, at a 1:20 dilution. Intravenous inoculation of volunteers was completed without complication, with all volunteers safely discharged at 1 hour post-CHMI following satisfactory observation. The overall safety profile was in-keeping with AEs as expected in *P. vivax* CHMI, and comparable to AEs as reported in previous *P. vivax* blood-stage challenge studies, as summarised below [92, 93].

Safety data was actively collected (solicited adverse events) on the following symptoms deemed indicative of *P. vivax* infection at post-challenge clinic visits until 28 days after CHMI: subjective feverishness, chills, rigors, sweats, headache, nausea, vomiting, diarrhoea, myalgia, arthralgia, lower back pain, fatigue and general malaise. Clinic visits were once or twice daily from day of challenge until completion of anti-malarial therapy, depending on the number of days since challenge and qPCR result, followed by assessment at 6 days post treatment initiation and 28 days post-CHMI, as set out by the study protocol. The severity of adverse events were self-assessed by volunteers who were counselled on criteria for grading as set out in Table 13. Unsolicted AEs and SAEs were assessed throughout the study period.

Prior to, or at the time of malaria diagnosis, the majority of volunteers (4/6) experienced either no or mild AEs, whilst 2/6 volunteers reported a constellation of moderate to severe AEs including feverishness, chills, sweats, headache, fatigue and malaise (Figure 3). AEs reported as moderate or severe in intensity were more frequent post-diagnosis, following initiation of anti-malarial therapy and were reported by 5/6 volunteers, each experiencing 1-6 AEs graded as severe in intensity. However, no AE reported as severe persisted at grade 3 for more than 48 hours, with the vast majority resolving completely within 24 hours (Table 4). The most frequent AEs reported as moderate-severe (grade 2-3) in intensity were feverishness, chills, malaise, fatigue and sweats (Figure 2). The large majority of all AEs resolved within 72 hours of anti-

malarial treatment, with only a single volunteer reporting any solicited AE in the period between two and six days after treatment of mild-moderate intensity, which completely resolved at 28 days after challenge.

AEs relating to physical observations were mostly mild in nature, with a single grade 3 pyrexia occurring (graded as per local SOP), which resolved within 24 hours (Figure 4). Of the laboratory AEs recorded, two were grade 3 in severity (graded as per local SOP), both relating to lymphocytopaenia in two volunteers at the time of diagnosis (Tables 4 and 5). These grade 3 events were transient, resolving completely within 6 days, and the abnormality was deemed to be in keeping with *P. vivax* malaria diagnosis. Given reported transaminitis in previous *P. vivax* CHMI studies, liver function tests were monitored through the post-CHMI period on days 7 and 14 post-challenge, on day of diagnosis, days 1 and 6 following treatment commencement and 28 days after challenge. Moderate (grade 2) transient derangement of ALT on liver function tests was seen in only 2/6 volunteers, detected at 6 days post-treatment. Both volunteers were asymptomatic and transaminases normalised by 28 days post-CHMI (within 10 days).

To supplement clinical assessment following initiation of anti-malarial therapy and confirm parasitological clearance, qPCR was performed on days 1 and 2 post-treatment. There was a substantial reduction in parasite genome copies/mL by qPCR in all volunteers within 48 hours of therapy (5/6 treated with artemether/lumefantrine (Riamet), 1/6 treated with atavoquone/proguanil (Malarone)) and qPCR remained negative at 6 days post-treatment initiation in all volunteers. AEs relating to treatment were infrequently reported, and were mild-moderate in nature where experienced (Figure 5). No grade 3 unsolicted AE was reported and there have been no SAEs to date in the VAC069 study.

Feasibility of CHMI through intravenous administration of the *P. vivax* inoculum was demonstrated by the successful infection of all volunteers, with a mean day of diagnosis of 15 days after CHMI (range 12.5-16.5). The two volunteers receiving the highest inoculum dose were diagnosed at days 12.5 and 15.5 respectively, those receiving a 1:5 dilution were both diagnosed at day 15 and those receiving the 1:20 dilution were diagnosed at days 15.5 and 16.5. All three dilutions were therefore demonstrated to be reliably infective. Based on these infectivity data, and accounting for possible variation in number of parasitised erythrocytes between thawed cryopreserved vials, an inoculum dilution of 1:10 (prepared as per Jenner laboratory SOP) will be utilised for *P. vivax* blood-stage CHMI in this VAC071 study.

Volunteers are scheduled to return to undergo a second challenge in September 2019 (approximately) and third challenge in February 2020 (approximately) to establish safety and feasibility of secondary and tertiary *P. vivax* controlled blood-stage challenge. The final follow-up will be 90 days after the tertiary challenge, approximately 1 and half years from enrolment. The natural immune response to primary, secondary and tertiary challenge, as well as gametocytaemia will also be explored following primary, secondary and tertiary blood-stage *P. vivax* infection. PCR quantification of gametocytes following primary challenge, as well as in-depth immunological analysis of the natural immune response, including human and parasite transcriptomics, are currently underway.

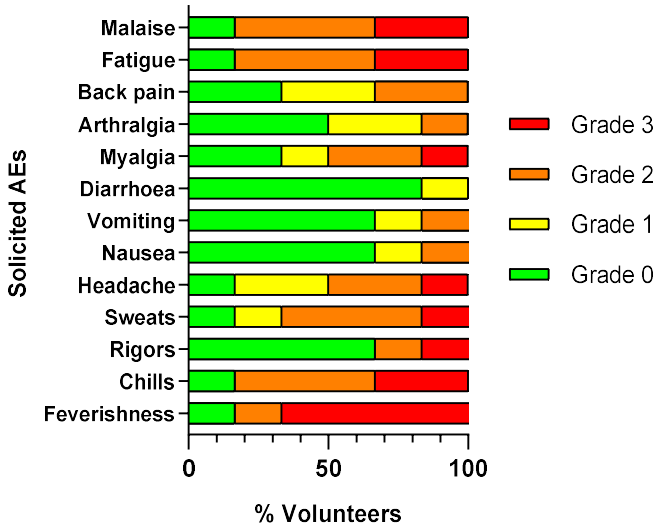

Figure 2: Maximal severity of solicited AEs following *P. vivax* blood-stage CHMI in the VAC069 study (n=6)

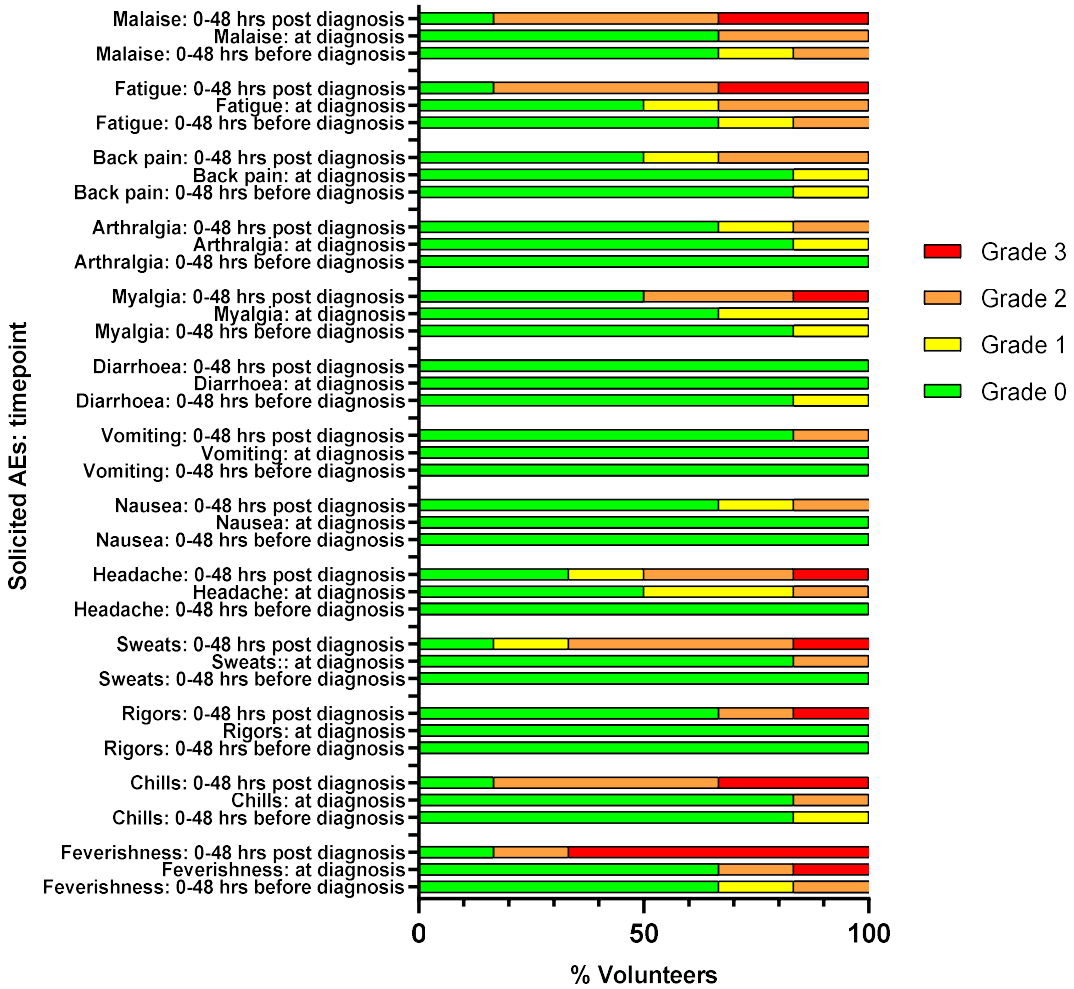

Figure 3: Maximal severity of solicited AEs reported by all volunteers (n=6) in the VAC069 study at 0-48 hours prior to diagnosis, on day of diagnosis and 0-48 hours post-diagnosis

Volunteer	Grade 3 AE	Timepoint	Persisted at grade 3	Resolution
6901002	Feverishness	T+1	24 hours	T+2
6901003	Feverishness	DoD/C+12.5	48 hours	T+2
	Chills	T+1	24 hours	T+2
	Sweats	T+1	24 hours	T+2
	Myalgia	T+1	24 hours	T+2
	Fatigue	T+1	24 hours	C+28
	Malaise	T+1	24 hours	T+2
6901006	Feverishness	T+1	24 hours	T+2
	Chills	T+1	24 hours	T+2
	Headache	T+1	24 hours	T+2
	Fatigue	T+1	24 hours	T+2
	Malaise	T+1	24 hours	T+2
6901009	Feverishness	T+1	24 hours	T+2
	Rigor	T+1	24 hours	T+2

Table 4: Onset and duration of grade 3 solicited AEs reported by all volunteers (n=6) in the VAC069 study

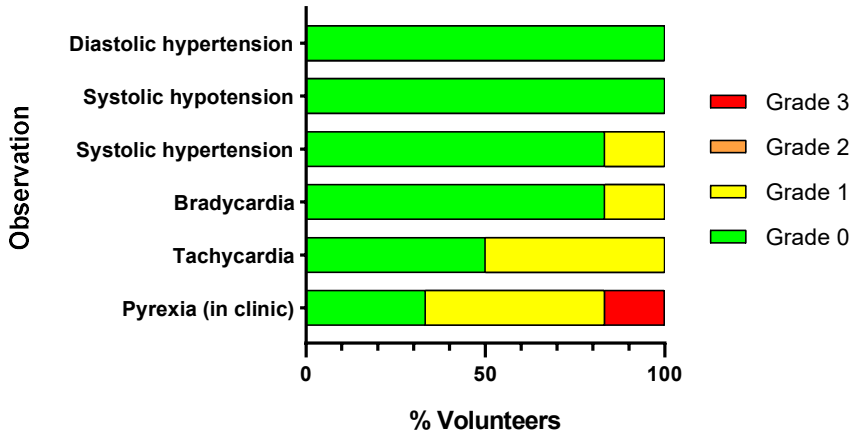

Figure 4: Percentage of all volunteers (n=6) with AEs in recorded physical observations

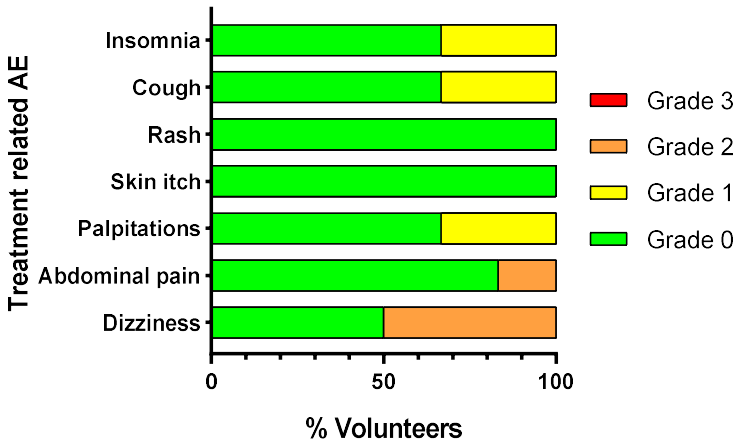

Figure 5: AEs possibly relating to treatment in all volunteers (n=6)

Laboratory AE	Volunteer	Timepoint(s)	Result	Grade	Action taken
Hyponatraemia	6901003	C+28	134	1	No clinical concern, no action taken
	6901003	C+90	134	1	No clinical concern, no action taken
Hypokalaemia	6901002	C+90	3.3	1	No clinical concern, no action taken
	6901005	DoD	3.3	1	Repeated within 24 hours at next visit, resolved (3.7)
	6901003	C+90	3.3	1	No clinical concern, no action taken
ALT	6901003	T+6	135	2	Asymptomatic, repeated in 48 hours (80 - grade 1) and then resolved at C+28 (21)
	6901006	T+6	56	1	Repeated and resolved (29) at next visit (C+28),
	6901007	T+6	80	1	Repeated and resolved (31) at next visit (C+28)
	6901009	T+6	114	2	Asymptomatic, repeated in 72 hours and resolved (40)

Table 4: Laboratory AEs in biochemistry profile (urea and electrolytes, liver function tests) in all volunteers (n=6) in the VAC069 study

Laboratory AE	Volunteer	Sex	Timepoint(s)	Result	Grade	Action taken
Anaemia	6901002	M	T+6	119	1	Repeated and resolved at next visit (C+28)
	6901005	F	C+16, C+34 (C+14-C+90)	98 (98-109)	(1-)2	Monitored from C+14-C+90, AE ongoing at C+90 (102 - grade 1). Referred to GP for ongoing medical care, with follow-up of AE ongoing.
Low white blood cell count	6901002	M	T+1	3.3	1	Consistent with *P. vivax* diagnosis, repeated and resolved (6.53) at next visit (T+6)
	6901003	F	T+1	2.93	1	Consistent with *P. vivax* diagnosis, repeated and resolved (6.86) at next visit (T+6)
	6901005	F	T+1 (C+7-C+90)	1.68 (1.68-3.39)	(1-)2	Monitored from C+7-C+90, AE ongoing at C+90 (3.36 - grade1). Referred to GP for ongoing medical care, with follow-up of AE ongoing.
	6901006	M	T+1 (C+14-T+1)	2.36	2	Monitored from C+14-T+6, resolved at T+6 (4.84) - consistent with *P. vivax* diagnosis
Thrombocytopaenia	6901003	F	T+1	114	2	Consistent with *P. vivax* diagnosis, repeated and resolved (233) at next visit (T+6)
	6901005	F	T+1(DoD-T+1)	115 (115-127)	(1-)2	Consistent with *P. vivax* diagnosis, repeated and resolved at T+6 (219)
Neutropaenia	6901005	F	C+45 (C+14-C+90)	0.76 (0.76-1.46)	(1-)2	Monitored from C+14-C+90, AE ongoing at C+90 (1.26 – grade 1). Referred to GP for ongoing medical care, with follow-up of AE ongoing.
Lymphocytopenia	6901002	M	T+1 (DoD-T+1)	0.72 (0.72-0.86)	(1-)2	Consistent with *P. vivax* diagnosis, repeated and resolved at T+6 (1.63)
	6901003	F	T+1	0.77	1	Consistent with *P. vivax* diagnosis, repeated and resolved (2.53) at T+6
	6901005	F	DoD (DoD-T+1)	0.44 (0.44-0.63)	(2-)3	Consistent with *P. vivax* diagnosis, repeated at resolved (1.63) at T+6
	6901006	M	T+1 (C+14-T+1)	0.3(0.3-0.83)	(1-)3	Consistent with *P. vivax* diagnosis, repeated and resolved at T+6 (2.07)

Table 5: Laboratory AEs in full blood count in all volunteers (n=6) in the VAC069 study

5.3 Conduct of CHMI trials

5.3.1 Standardisation of CHMI studies

Following a collaborative consensus process involving investigators from the USMMVP, Sanaria, University of Maryland, University of Oxford, RUNMC, 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 [78]. There is no equivalent document for controlled blood-stage infection or for *P. vivax* challenge, however, this study will be conducted in line with the following key points.

- All volunteers should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination, pregnancy test for female volunteers.
- Volunteers should be questioned about the occurrence of adverse events and use of medication at each follow-up visit.
- In the event that a volunteer does not attend a scheduled follow-up visit it is imperative that Investigators find that volunteer as quickly as possible and assess them for patent parasitaemia and clinical malaria. Should the volunteer withdraw consent from further follow-up prior to receipt of antimalarial drugs, it may be appropriate to withdraw the volunteer from the trial protocol and administer a course of antimalarial 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 least once daily and at any subsequent visits for medical attention. Directed physical examination should be performed when necessary.
- It is critical that every volunteer 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 volunteers closely to ensure compliance with the treatment regimen.
- After challenge, all volunteers should be followed until they have completely finished anti-malaria treatment.
- Volunteers 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 volunteer or volunteer group. While safety monitoring committees are not a requirement for Phase I trials, they should be considered a requirement for CHMI trials which have an efficacy/human challenge component and which have major potential safety concerns.

5.3.2 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 volunteer 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 review by Moorthy from 2011, and include; [78]

1. Volunteer 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 ICH and/or WHO Good Clinical Practice Guidelines with the aim of maximising scientific benefit whilst minimising risk.
4. The raw data (both microscopy and PCR where available) from challenge trial datasets should be made publicly available to facilitate scientific benefit to the community.
5. If an unexpected 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.

5.3.3 Clinical presentation post-CHMI

Nearly all unvaccinated volunteers in CHMI studies develop symptoms of clinical malaria infection; based on data predominately from *P. falciparum* challenge studies approximately one-fifth of volunteers temporarily develop symptoms graded as severe (symptoms that prevent daily activities), but severe or life-threatening malaria has never occurred [96]. The expected time-course, symptoms and management of clinical malaria are outlined in Section 9.

Routine laboratory checks generally show a moderate decrease in leukocyte and platelet numbers during infection, with no change in haemoglobin concentration [97]. Bleeding or thrombogenic complications have never been described [96, 97]. Abnormalities of liver enzymes have been observed, but these abnormalities have rarely resulted in clinical manifestations (just one volunteer with raised ALT associated with abdominal pain and vomiting in the recent RCT from Cali [27]) and they resolved after a few days.

It is estimated that human malaria challenge infections have now been conducted with *P. falciparum* in over 2,650 volunteers and in over 300 volunteers with *P. vivax* [98]. In 2009, safety concerns were raised when young volunteer suffered a cardiac event shortly after treatment for diagnosed malaria, following a *P. falciparum* challenge. This 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 [99], it has been generally agreed that volunteers with an increased risk of cardiac disease should be excluded from such trials [78]. A further case of myopericarditis has since been identified in a recent *P. falciparum* CHMI study, also at the Nijmegen, Netherlands centre, but in this case the individual was also diagnosed with an intercurrent rhinovirus infection so that the relation to malaria infection is again uncertain. There was a brief episode of clinical chest pain and the volunteer made a full recovery [100].

5.3.4 Oxford's experience conducting CHMI trials

The University of Oxford has been conducting CHMI studies (with *P. falciparum*) for the last 18 years. To date, nearly 600 volunteers have undergone CHMI in studies conducted by the University of Oxford, including more than 180 unvaccinated volunteers. An analysis has shown that the symptoms of malaria experienced by volunteers undergoing sporozoite challenge by mosquito bite in Oxford are broadly similar to those experienced by volunteers challenged by mosquito bite at Radboud University Nijmegen Medical Center (RUNMC) and the US Military Malaria Vaccine Program (USMMVP) [101]. In Oxford, four volunteers amongst nearly 600 individual challengees have required admission to hospital for observation following challenge (SAE related to CHMI). All of these subjects made a complete recovery. This will be only the

second blood-stage *P. vivax* challenge study conducted in Oxford, but we have based our study design on that of the previous *P. vivax* blood-stage challenge studies in Australia, combined with our own extensive experience of *P. falciparum* sporozoite challenge studies and our recent VAC068 *P. vivax* sporozoite challenge study.

In one Phase I/IIa sporozoite challenge study assessing the efficacy of viral vectored malaria vaccines in Oxford (VAC039; Clinicaltrials.gov reference: NCT01142765), a volunteer who underwent sporozoite challenge on 1st October 2010 failed to attend his next scheduled study visit on 7th October 2010 [102]. The police were immediately informed and began a nationwide search for the individual. All volunteers had been informed at screening that the police would be notified should they go missing following CHMI if they had not completed a full course of an appropriate anti-malarial treatment. The volunteer was found in the Netherlands by the local police 17 days following CHMI. He then had very mild malaria symptoms. He was admitted to a local hospital where he received appropriate treatment for *P. falciparum.* He had no signs of severe malaria but showed an altered mental state considered unrelated to malaria with apparent memory loss and suicidal ideation. He was therefore transferred for in-patient psychiatric assessment and discharged a few days later to his GP's care. He has subsequently been reportedly diagnosed as schizophrenic.

It emerged that from 2nd October 2010, the day after CHMI, the volunteer had experienced an alteration in his expected behaviour following his arrest the previous evening by the police relating to their investigation of a serious crime, of which he was later convicted. This arrest appeared to trigger his leaving home and disappearance and appears relevant to the subsequent finding of memory loss and suicidal ideation. It emerged on subsequent investigation that the volunteer actually had a history of some psychiatric morbidity pre-dating his involvement in the study by many years, which was not disclosed at screening by the volunteer or his GP. Of note, the volunteer had attended 9 clinic visits prior to challenge and appeared a reliable and appropriate volunteer.

This event was extensively discussed with investigators, colleagues and appropriate authorities and non-study related causality agreed. Given the exceptional circumstances relating to this case, it seems very unlikely that a similar event would happen again in the future. Management of the event was extensively reviewed by the trial's sponsor, the MHRA and ethical committee who felt that appropriate and timely action was taken. Follow-up procedures following CHMI have been reviewed locally, and it has been decided that for future studies volunteers should be contacted daily on days 1-5 post sporozoite challenge in order to make sure they are contactable and well. Daily telephone contact will similarly be conducted in this study on days 1-6, before daily clinic visits commence on day 7.

5.3.5 Conducting CHMI in the context of the COVID-19 pandemic

Due to the COVID-19 pandemic this trial was temporarily halted in March 2020. The incidence of COVID-19 continues to change in England and some ongoing transmission is likely to remain when this trial restarts in 2021 [103]. This trial will only proceed when rates of COVID-19 infections in England are low and the risk of contracting COVID-19 during malaria challenge is small, however the risk cannot be eliminated. There is currently no evidence for the possible effects of co-infection with malaria and COVID-19. As the risk of co-infection cannot be quantified, participants will be tested for COVID-19 prior to malaria challenge and any participant that tests positive for COVID-19 will not proceed with malaria challenge.

Participants who test positive for COVID-19 will be advised to self-isolate as per Public Health England guidance and directed to local NHS services for further care if required. Positive COVID-19 PCR results will be reported as a notifiable disease as per Public Health England guidance.

Fevers which occur following malaria challenge may pose diagnostic difficulties as concurrent COVID-19 infection will likely remain an ongoing possibility in England. Data from the 12 primary and 4 secondary CHMI with *P. vivax* conducted in Oxford to date, show that no participants developed symptoms that are consistent with malaria within the first week post challenge. Pyrexia or subjective feelings of feverishness occurred in only half of the participants prior to diagnosis and treatment of malaria. However the majority of remaining participants who were initially apyrexial, did subsequently develop fever after commencing malaria treatment, although this was short lived and resolved within 2 days. Following malaria challenge, participants first developed pyrexia or subjective feelings of feverishness between days 10 to 20 post challenge (median 14 days), with corresponding malaria qPCR levels of 100 to 9600 genome copies/mL (median 2700 genome copies/mL). No recorded pyrexia of >37.8°C occurred at a malaria qPCR below 1000 genome copies/mL in any participant. Malaria diagnosis was made in participants within a few days following the onset of symptoms with some participants reporting no symptoms prior to laboratory diagnosis. Participants were diagnosed with malaria based on a combination of clinical symptoms, malaria qPCR and thick film microscopy between days 12 and 21 post challenge (median 15.5 days).

Fevers within the first week after malaria challenge are therefore unlikely to be due to malaria. After the first week post challenge, malaria as the cause of fever becomes increasingly likely with increasing time since challenge. However, as concurrent COVID-19 infection will likely remain a possibility, testing for COVID-19 has been added in the post-challenge period for participants who become febrile. For participant safety, if a participant tests positive for COVID-19 prior to malaria diagnosis, they will be treated for malaria regardless of if they have reached the criteria for malaria diagnosis.

There is no evidence for any detrimental effect of anti-malarial medication on the outcome of concurrent COVID-19 infection. This is in the context of widespread antimalarial use during the COVID-19 pandemic in Africa, where no safety signal has emerged. Recent randomised controlled trials have shown no benefit of Hydroxychloroquine in COVID-19 [104], but also no harm, and Chloroquine will not be used for malaria treatment in this trial. The anti-malarial medications Riamet and Malarone are not expected to have any effects on the outcome of COVID-19 disease.

Social distancing requirements to control COVID-19 will likely remain in place to some extent in England. To abide with these, participants' attendances at study clinics will be carefully planned to adhere to social distancing guidelines. Participant flow will be arranged so that contact with other people is minimised and Personal Protective Equipment (PPE - compromising apron, gloves, surgical mask and eye protection) will be worn by members of clinical staff for all participant visits. This will follow an infection control SOP that covers all PPE requirements for clinical trials at the Oxford Vaccine Centre in the era of the COVID-19 pandemic. Any clinic visits that are carried out on a participant that is self-isolating will be conducted in a separate clinical area with side access to avoid any contact with other volunteers.

As the national rollout of COVID-19 vaccines proceeds, some participants may be offered a COVID-19 vaccine around the time of planned malaria challenge. It is unknown what effect concomitant COVID-19 vaccine administration around the time of CHMI may have on the immune response to malaria infection, which is being studied in this trial. In addition reactogenicity from COVID-19 vaccination may be difficult to distinguish from symptoms of malaria and may lead to earlier diagnosis and treatment of malaria after challenge. Therefore concomitant COVID-19 vaccination around the time of CHMI has been added as an exclusion criteria in this trial.

6 INVESTIGATIONAL PRODUCTS

6.1 PvDBPII

PvDBPII was manufactured under Good Manufacturing Practice (GMP) conditions by Syngene International, Bangalore, India, and the drug product was vialed and released by Zydus Cadila, Ahmedabad, India.

PvDBPII is supplied as a liquid at a concentration of 200 µg/mL in sterile aliquots in 0.5 mL clear glass vials. Further details relating to batch release and manufacturing can be found in the PvDBPII IND.

All vaccines will be certified for release by a qualified person (QP) at the CBF, University of Oxford.

6.2 Matrix M1

Matrix M1 is manufactured under Good Manufacturing Practice conditions by Novovax AB (Uppsala, Sweden). Matrix-M1 will be supplied to the clinical site by Novovax AB and the adjuvant will be labelled for investigational use only.

Matrix-M1 is formulated at a concentration of 0.375mg/ml in PBS. The drug product is filled into sterile 3mL glass vials. Matrix-M1 (85 parts Matrix A and 15 parts of Matrix C) is obtained by simply mixing Matrix A and C, followed by dilution in PBS, filtration though filter 0.22 µm and filling into vials in a volume of 2 ml. Matrix-M1 is a colourless slightly-opalescent non-viscous liquid.

Final batch certification and associated labelling takes place at the CBF, University of Oxford. Further details relating to batch release and manufacturing can be found in the Matrix M1 IMP-D.

6.3 Storage of Vaccines

All vaccines will be stored between –70°C and –90°C. All movements of the study vaccines will be documented. Vaccine accountability, storage, shipment and handling will be in accordance local SOPs.

Matrix-M1 is stored refrigerated at 2 to 8°C and protected from light. All movements of the adjuvant will be documented. Accountability, storage, shipment and handling of Matrix-M1 will be in accordance with relevant local SOPs and forms.

6.4 Administration of Vaccines

All vaccines will be administered intramuscularly in the deltoid muscle of the arm, according to SOP VC002 Vaccination. The vaccinating investigator will wear gloves and eye protection. During administration of the vaccines, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. On vaccination day, vaccines will be allowed to thaw to room temperature and administered within 1 hour.

For each immunization, one vial of PvDBPII will be used per subject. One vial of adjuvant will be used for a maximum of two immunizations. A mixture of PvDBPII at a dose of 50 µg, with 50µg of Matrix-M1 will be administered to all volunteers. Matrix-M1 and PvDBPII will be mixed at the bedside immediately prior to administration. Any vaccine not administered within the time frame will be destroyed.

7 OBJECTIVES AND ENDPOINTS

7.1 Primary Objectives

To assess the safety and tolerability of the PvDBPII vaccine formulated in Matrix M1

To establish whether the PvDBPII-Matrix M1 vaccine can demonstrate a reduced parasite multiplication rate in vaccinated subjects compared to infectivity controls in a blood-stage controlled human malaria infection model

7.1.1 Primary Safety Outcome Measures

The specific endpoints for safety and reactogenicity will be actively and passively collected data on adverse events. The following parameters will be assessed:

- Occurrence of solicited local reactogenicity signs and symptoms for 7 days following each vaccination
- Occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of unsolicited adverse events for 28 days following the vaccination
- Change from baseline for safety laboratory measures for 28 days following vaccination
- Occurrence of serious adverse events during the whole study duration

Solicited and unsolicited AE data will be collected at each clinic visit. It will be collected from diary cards, clinical review, clinical examination (including observations) and laboratory results. This AE data will be tabulated and frequency, duration and severity of AEs compared to the AE data collected for the infectivity control group undergoing parallel CHMI recruited as part of the VAC069 clinical trial. Hematological and biochemical laboratory values will be presented according to local grading scales and tabulated by group, for comparison with the laboratory AE data collected for infectivity control groups undergoing CHMI in parallel.

SAEs, AEs of special interest and withdrawal due to AE(s)/SAE(s) will be described in detail.

The vaccinated volunteers will be followed for approximately 1 year following their first vaccination with PvDBPII-Matrix M1.

7.1.2 Primary Efficacy Outcome Measures

Quantitative PCR-derived parasite multiplication rate (PMR) will be the primary efficacy endpoint and a comparison of the endpoint between the volunteers vaccinated with PvDBPII-Matrix M1 and malaria-naïve controls partaking in parallel CHMI will constitute the primary analysis for efficacy.

The arithmetic mean of the three replicate PCR results obtained for each individual at each timepoint will be used for model-fitting. Negative individual replicates will be handled as specified in the Jenner Institute qPCR SOP. qPCR data points which, based upon the mean of the three replicates, are negative or below the limit of quantification will be handled as specified in the Jenner Institute qPCR SOP. PMR will be calculated using a linear model fitted to log10-transformed qPCR data.

The distribution of PMRs will be assessed for deviation from normality visually and using a d'Agostino-Pearson test. Standard ladder-of-power transformations may be applied to achieve normality. Equality of variance will be tested with an F test. In the event of deviation from normality, PMRs will be compared by two-tailed Mann-Whitney test; otherwise a two-tailed two-

sample t-test will be used, with Welch's correction if variances are non-equal as assessed by F-test.

7.2 Secondary Objectives

To assess the humoral and cellular immunogenicity of the PvDBPII-Matrix M1 vaccine candidate.

To assess immunological readouts for association with a reduced parasite multiplication rate

To assess the durability of any reduction in PMR in Group 3 volunteers who undergo a fourth vaccination followed by re-challenge approximately 5 months after the primary challenge

7.2.2 Secondary Immunological Outcome Measures

PvDBPII-specific immunogenicity will be assessed by a variety of immunological assays, with comparison before and after vaccination. The main outcome measures will be humoral and B cell responses to the *P. vivax* Duffy-binding protein (PvDBPII) – total IgG, isotypes and avidity; T cell responses to PvDBPII by flow cytometry assays and *in vitro* functional PvDBPII inhibitory binding assays. Other established and exploratory immunology assays may be carried out, including through collaboration with other specialist laboratories, which may be outside of UK/Europe. This would involve transfer of serum/plasma, but samples would be anonymised. Volunteers will be consented for this.

The relationship between PMR in vaccinated subjects and anti-PvDBPII antibody responses induced by the PvDBPII-Matrix M1 vaccine will also be assessed.

Any other immunological analyses performed will be reported as not pre-specified in the trial protocol. Other analyses may be detailed in the VAC079 laboratory plan. Some assays may be duplicated at different sites. Some of these will involve analysis of frozen samples, and others analysis of fresh samples.

7.2.3 Secondary Efficacy Outcome Measures

Quantitative PCR-derived parasite multiplication rate (PMR) will be the secondary efficacy endpoint for assessing the durability of vaccine induced reduction in PMR. For this secondary efficacy analysis comparision of the PMR will be made between volunteers in Group 3 who received 4 vaccinations of PvDBPII-Matrix M1 and two control groups: malaria-naïve controls undergoing primary CHMI and volunteers undergoing their second CHMI in the parallel VAC069 study. PMRs will be calculated from qPCR data using the same method as above for the primary efficacy analysis.

8 DESCRIPTION AND JUSTIFICATION OF STUDY DESIGN

8.1 Study rationale

As outlined above, the available methods for preventing and treating infections with *P. vivax* in the field are inadequate. An effective vaccine against *P. vivax* would contribute significantly to disease control and elimination efforts. Currently, only two *P. vivax* candidate vaccines, both targeting the pre-erythrocytic stage, have progressed to Phase II studies. CHMI efficacy data for only one of these candidates, VMP001/AS01$_B$, is currently published and whilst the vaccine was well-tolerated and immunogenic, there was no efficacy following sporozoite challenge.

The PvDBPII vaccine candidate, formulated with the adjuvant GLA-SE, has been demonstrated to be safe, tolerable and immunogenic in a single blinded, dose-escalation Phase Ia study [29]. Progression of this promising candidate vaccine into this Phase IIa challenge study will be the first trial of efficacy of a *P. vivax* blood-stage vaccine candidate. Even if partially efficacious, through a reduced parasite multiplication rate, this could represent an important tool reducing the *P. vivax* global health burden and moves towards elimination. Assessment of efficacy in this study will therefore inform future progression of these novel vaccines into further clinical testing into larger, but more expensive and logistically demanding trials, in endemic settings.

8.2 Study Overview

This is an open-label, single-centre first-in-human Phase I/ IIa *P. vivax* blood-stage CHMI trial to assess the safety, immunogenicity and efficacy of the candidate malaria vaccine PvDBPII formulated in adjuvant Matrix M1.

Up to 24 healthy, malaria-naïve adults aged between 18 and 45 years will be recruited at the CCVTM, Oxford. Volunteers in Groups 1 and 2 will receive three doses of the PvDBPII-Matrix M1 vaccine intramuscularly. Volunteers in Group 1 who complete 3 vaccinations and CHMI, will be invited back for a fouth vaccination at 5 months following their third vaccination and form a new study group - Group 3.

Between two to four weeks post-boost, all volunteers will undergo *P. vivax* blood-stage CHMI, induced by injection of *P. vivax* infected erythrocytes. Volunteers will have blood taken at regular intervals following vaccination and in the post-CHMI period to assess the immune response to vaccination and subsequent challenge, as well as parasite growth dynamics and gametocytaemia. Close monitoring will continue until volunteers meet criteria for treatment or until 28 days after challenge in Group 1 or until 21 days in Groups 2 and 3, when treatment will be started empirically.

Therapy will be with either Riamet or Malarone. As infection will be mediated via intravenous injection of blood-stage parasites, there will be no liver-stage infection and no hypnozoite formation, thereby eliminating need for radical cure with primaquine therapy.

During each CHMI period, a matched number of malaria-naïve infectivity controls, recruited as part of the VAC069 study, will be challenged in parallel.

All vaccinations, follow-up visits, CHMI and follow-up in the post-challenge period will be performed at the CCVTM in Oxford. Follow-up will be for 9 months after the final vaccination.

8.3 Study groups

This study was originally planned with one study group, Group 1, comprising 10-12 volunteers, who were planned to receive the same doses of the PvDBPII-Matrix M1 vaccine at 4 weekly intervals and be challenged with malaria at the same time.

However this trial was temporarily halted in March 2020 due to the COVID-19 pandemic. 12 volunteers were enrolled at the start of 2020 and 11 volunteers had completed two vaccinations

of the PvDBPII-Matrix M1 vaccine and were due their third and final vaccination at the end of March 2020, which has been delayed. All currently enrolled volunteers who consent to continue in the study, will now complete their third and final vaccination at 12 to 18 months and undergo CHMI 2 to 4 weeks after their last vaccination. These volunteers will be followed up for 9 months from the time of their last vaccination, approximately 2 years from enrolment.

If fewer than 8 volunteers from Group 1 return to complete all three vaccinations followed by CHMI, then new volunteers will be recruited into Group 2, to make up a total of 10 to 12 volunteers between Groups 1 and 2 who complete three vaccinations and CHMI. Group 2 volunteers will undergo 3 vaccinations at the original 4 weekly intervals, followed by CHMI 2 to 4 weeks after the last vaccination. They will be followed up for 12 months from enrolment.

Volunteers who have completed 3 vaccinations and CHMI in Group 1 will be invited at one of their post challenge follow-up visits to return for a fourth vaccination 5 months after their third vaccination, followed by secondary CHMI 2-4 weeks after the last vaccination and will form Group 3. These volunteers will be followed up for 9 months from the time of their last vaccination, approximately 2.5 years from initial enrolment.

First volunteers:

The first volunteer to receive a dose of PvDBPII-Matrix M1 will be vaccinated alone and then reviewed on the day following vaccination. Providing there are no safety concerns at the Day 1 review, or recorded in the diary over the next 24 hours, another two volunteers may be immunised a minimum of 2 days after the first volunteer, at least one hour apart. Following review of the second and third volunteers at the 7 day timepoint, an internal safety review will be conducted. If no safety concerns are identified, the remaining volunteers in the group will be vaccinated.

8.3.1 Control groups during challenge

Malaria-naïve controls for CHMI, will be recruited as part of the currently ongoing VAC069 study, a *P. vivax* challenge study to assess safety and feasibility of the blood-stage *P. vivax* challenge model, and to assess the effect of previous challenge on subsequent homologous re-challenge. Malaria-naïve controls recruited in the VAC069 study will undergo primary challenge in parallel with VAC079 volunteers. The data generated from the challenge of malaria-naïve controls within the VAC069 study will be utilised on a de-identified basis, for comparative analysis with challenge of VAC079 vaccinees for assessment of vaccine efficacy.

Group	Group size	D0	D28	12-18 months	14-28 days post 3rd vaccination
Group 1	10-12	PvDBPII 50ug Matrix M1 50ug	PvDBPII 50ug Matrix M1 50ug	PvDBPII 50ug Matrix M1 50ug	CHMI

Table 6a: Study Group 1

Group	Group size	D0	D28	D56	D70-84
Group 2	Up to 12	PvDBPII 50ug Matrix M1 50ug	PvDBPII 50ug Matrix M1 50ug	PvDBPII 50ug Matrix M1 50ug	CHMI

Table 6b: Study Group 2. *New participants will only to be recruited into Group 2, if fewer than 8 participants in Group 1 complete all 3 vaccinations and CHMI, to make up a total number of 10 to 12 participants between Groups 1 and 2.*

Group	Group size	D0	D28	12-18 months	14-28 days post 3rd vaccination	5 months post 3rd vaccination	14-28 days post 4th vaccination
Group 3 (subset of Group 1)	Up to 6	PvDBPII 50ug Matrix M1 50ug	PvDBPII 50ug Matrix M1 50ug	PvDBPII 50ug Matrix M1 50ug	CHMI	PvDBPII 50ug Matrix M1 50ug	Second CHMI

Table 6c: Study Group 3. *Subset of Group 1 volunteers who consent to return for fourth vaccination, followed by second CHMI– all activity in this table up to and including CHMI post 3rd vaccination is undertaken as part of Group 1.*

8.4 Duration of study

8.4.1 Definition of the start and end of the trial

The start of the trial is defined as the date of enrolment of the first volunteer. The end of the trial is the date of the last follow-up of the last volunteer.

8.4.2 Duration of volunteer participation

Volunteers will be considered to be enrolled in the trial on the receipt of the first vaccination. Total duration of follow-up will be approximately 2 years from enrolment for Group 1 volunteers, extended by about 1 year due to the temporary trial halt. Group 2 volunteers will be followed up for 1 year from enrolment. Group 3 volunteers will be followed up for 2.5 years from initial enrolment.

8.5 Potential risks for volunteers

8.5.1 Phlebotomy

The maximum volume of blood drawn over the study period should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur. The actual total blood volume will relate to the timing of malaria diagnosis and treatment during challenge.

Male regular blood donors may donate one unit (470mL) every 12 weeks and females every 18 weeks. However, a recent 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 470 mL every 8 weeks in male participant group and every 12 weeks in females) over 2 years. 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 96mL 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 maximum total blood volume drawn over 1 year in this study will be 1752mL in those participants originally in Group 1 who undergo a fourth vaccination and re-challenge in Group 3. For male participants, this is 1303mL less than the 8-week interval group in the NHSBT study and for females, 285mL less than the 12-week interval group, over the 1 year period. Therefore, we are confident that this volume should not compromise our volunteers. However, the additional effect of malarial infection on potential anaemia is acknowledged. For this reason, the full blood count will be monitored throughout the study period. As an additional precaution, volunteers weighing less than 50Kg at screening will be excluded from participation.

8.5.2 Vaccinations

Foreseeable risks from vaccination, which include local and systemic reactions, are specific to each IMP and full details available in the respective IBs.

As with any vaccine, Guillain-Barré syndrome or immune-mediated reactions that can lead to organ damage including serious allergic reactions may occur but this should be extremely rare. Serious allergic reactions including anaphylaxis could also occur and for this reason volunteers will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any serious adverse reactions.

Based on the safety data available from the 27 volunteers who received PvDBPII vaccine formulated in GLA-SE in the Phase Ia study [29], as well as data available from more than 1132 individuals who have previously received vaccines formulated in Matrix M1, the below adverse event profile is expected following vaccination with PvDBPII-Matrix M1:

Local adverse events such as injection site pain would be foreseeable to occur frequently. Less frequent local adverse events are likely to include erythema, swelling and bruising. Local AEs are likely to be mild in nature and should resolve rapidly, although there is the possibility of moderate or severe arm pain in some cases.

Among the solicited systemic complaints following Matrix M1-adjuvanted vaccines, headache, muscle pain, fatigue, chills, joint pain, wheezing, and eyelid swelling all occurred at higher rates among adjuvanted vaccinees, when compared to placebo vaccinees at an excess of ≥ 1% of subjects.

8.5.3 Risk of Infection with Blood Borne Organisms

This controlled blood-stage challenge will involve a blood transfusion, albeit of very small volume relative to standard blood transfusion. As for any transfusion, the risk of transmission of a blood borne infection cannot be completely eliminated. However, in order to minimise the risk, extensive screening for blood borne infections has been performed in the direct blood donor of the bank, as well as the initial source patient in Thailand, who provided blood for the mosquito infection by direct membrane feeding. 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. The source patient was screened for antibodies to *W. bancrofti* at the Thai field site.

In addition, the blood bank has been subjected to direct screening and tested negative for bacterial contamination, sterility testing, endotoxin screen and mycoplasma specific culture. Plasma derived from the bank has also been screened for blood borne infections, as detailed in Section 5.2.6 and has tested negative for all blood-borne infection screens by PCR. Further to this, as outlined in section 5.2.5, volunteers also underwent repeat serological testing for HIV, hepatitis B and C, syphilis, HITLV-1 and HTLV-2, 90 days after challenge was undertaken, to ensure that no seroconversion from a recently-acquired infection (that may have been

undetectable around the time of challenge and therefore undetectable in the bank) had occurred since the challenge period. All tests remained negative.

For additional safety assessment of blood-stage CHMI by intravenous injection of this new source of cyropreserved, infected erythrocytes, repeat blood-borne viral screening (HIV, hepatitis B and C, plus EBV and CMV, if negative at screening) will also be performed for all recipients of the inoculum at 96 days post-challenge, assessing for any change in serostatus post-exposure.

Since the blood donor has been resident in the UK or continental Europe in countries 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 is reduced by leukodepletion, as 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 [105]. The risk of infection is further reduced by the very small size of the inoculum. The volume to be transfused for this study will be at least nine hundred times smaller than the amount received in typical blood transfusion. Serum from volunteers will be collected before and after challenge for storage.

8.5.4 *Plasmodium vivax* infection

Volunteers are likely to develop symptomatic malaria infection following CHMI. Symptoms and signs will include feverishness, fever, tachycardia, hypotension, chills, rigors, sweats, headache, anorexia, nausea, vomiting, diarrhoea, myalgia, arthralgia, low back pain, thrombocytopenia and lymphopenia [101]. Unmonitored and untreated, *P. vivax* infection can be serious (but rarely fatal) and, for this reason, volunteers 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 volunteers in previous *P. vivax* blood-stage and sporozoite-stage CHMI studies have temporarily required intravenous fluid therapy for nausea and vomiting prior to treatment [27, 93]. If the Investigator judges the volunteer to be unwell enough to require intravenous fluids or continuous medical input, they shall be transferred to the John Radcliffe Hospital under the care of the NHS Infectious Diseases team until they are well enough to be discharged home.

With regards to the above, it is relevant to note that safety and parasitological data from 128 malaria-naïve subjects participating in CHMI studies in Oxford and the Netherlands were analysed and compared to a report from the US Military Malaria Vaccine Program. The authors found that cohorts with a longer pre-patent period or a higher peak parasitaemia did not consistently show a higher frequency of adverse events [106]. However, these data were on *P. falciparum* challenge, and there is a lot less experience with *P. vivax* challenge.

8.5.5 Medications dispensed to volunteers in course of trial

(a) Treatment of *Plasmodium vivax* Infection

P. vivax malaria infection will be treated with oral Riamet or oral Malarone.

Riamet contains 20mg of artemether and 120mg lumefantrine per tablet. 6 doses of 4 tablets will be given; the first dose is followed by additional doses after 8, 24, 36, 48 and 60 hours. If there is a contraindication to Riamet, oral Malarone will be used (see Summary of Material Product Characteristics (SmPC) for side effects of, and contraindications to Riamet and Malarone). At a minimum two doses (the first and third doses) of Riamet will be directly observed. The first doses will be observed in clinic and the volunteers will not return home until they are seen to be tolerating the medication.

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. Volunteers will be counselled that certain side effects, for example dizziness, may impact on the performance of skilled tasks such as driving.

As Riamet may increase the QT interval, Riamet will not be administered to volunteers 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. Volunteers will be advised to avoid grapefruit juice whilst taking Riamet as it can affect the bioavailability of the artemether [107]. Riamet is also contraindicated in volunteers using concomitant medications affecting the QT interval, and will not be given to these volunteers. Concomitant use of Riamet may decrease effectiveness of hormonal contraceptives.

Women using hormonal contraceptives will be advised to use an effective additional method of contraception whilst on Riamet treatment, until the start of the next menstruation after treatment.

Malarone contains 100 mg proguanil hydrochloride and 250 mg atovaquone per tablet. 4 tablets will be given once daily for 3 days. The first and second dose, out of the three doses of Malarone, will be directly observed in clinic. Prior to starting Malarone, volunteers will be screened for drug interactions and contraindications (including a serum β-hCG test in female volunteers).

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.

(b) Treatment of Symptoms Associated with Malaria

Volunteers will be dispensed cyclizine and paracetamol for the treatment of symptoms associated with malaria unless there are contraindications to these medications. Cyclizine is generally well tolerated, however, there is a risk of an allergic reaction. Other side effects include skin rashes or itching, drowsiness, headache, dry mouth, nose or throat, blurred vision, palpitations, difficulty urinating, constipation, anxiety, insomnia or hallucinations. Rare side effects include hypotonia, seizures, dizziness, hypertension, paraesthesia, jaundice, hepatitis, confusion or dyskinesias. Participants may be dispensed an appropriate, licensed alternative anti-emetic to cyclizine if they are unable to take cyclizine. Paracetamol is generally well tolerated, however, it can cause an allergic reaction or rarely pancytopaenia.

8.5.6 Risk of reaction to the blood sample

The donor of the blood used in the inoculum is Blood Group O and Rhesus (Rh) and Kell (K) negative. People with Blood Group O negative blood are considered 'universal donors', as recipients of their blood are unlikely to develop red cell alloantibodies when given much larger volumes of blood than is envisaged here. For women of child-bearing potential, transfusion of Rh and Kell negative blood is also essential to prevent sensitisation and the future risk of haemolytic disease of the newborn. In the case of Kell sensitisation, anti-K may additionally lead to suppression of red cell production in the fetus. The maximum volume of blood to be used in this study (0.025mL – 0.1mL packed red cells) is much smaller than that given in transfusion of one unit packed red cells (470mL). The risk of a transfusion reaction or development of antibodies to donor red cells, which may make blood transfusion more difficult in the future, cannot be completely excluded but is considered highly unlikely, both because of the small volume of blood used and because the blood has been leukodepleted. Nevertheless, the volunteers will be monitored for this possibility in the period immediately after the administration of the malaria parasite dose. A baseline set of observations (respiratory rate, heart rate, temperature and blood

pressure) will be taken before the administration of the inoculum. Volunteers will be reviewed 15 minutes (+/- 5 minutes) and 1 hour (+/- 20 minutes) after receiving the inoculum, with a repeat set of observations at each time point, before they are able to leave the CCVTM. The serum collected for storage (as described above) could be used to test for antibodies at a later date if deemed necessary.

8.6 Potential benefits for volunteers

Volunteers will not benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective *P. vivax* malaria vaccine regimen. Volunteers will also receive information about their general health status.

9 RECRUITMENT AND WITHDRAWAL FOR TRIAL VOLUNTEERS

Volunteers may be recruited by use of an advertisement +/- registration form formally approved by the ethics committee(s) and distributed or posted in the following places:

- In public places, including buses and trains, with the agreement of the owner / proprietor.
- In newspapers or other literature for circulation.
- On a website operated by the Investigators' clinical trials group or with the agreement of the owner or operator (including on-line recruitment through our website).
- As a post on a Twitter or Facebook or other similar account owned and operated by the Investigators' clinical trials group.
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation.
- By email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the Oxford Vaccine Centre.
- On stalls or stands at exhibitions or fairs.
- Via presentations (e.g. presentations at lectures or invited seminars).
- Oxford Vaccine Centre databases: We may contact individuals from databases of groups within the CCVTM (including the Oxford Vaccine Centre database) of previous trial participants who have expressed an interest in receiving information about all future studies for which they may be eligible.

Volunteers enrolled in Group 1 will be asked at one of their post-primary CHMI follow up visits whether they would be interested in enrolment into Group 3 and undergo a fourth vaccination and repeat challenge. Those who are will be re-consented at or after their C+96 visit, prior to enrolment into Group 3, which will occur at the fourth vaccination visit.

9.1 Informed consent

9.1.1 Informed consent

The information sheet will be made available to the volunteer at least 24 hours prior to the screening visit. At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following will be emphasised:

- Participation in the study is entirely voluntary.
- Refusal to participate involves no penalty or loss of medical benefits.
- The volunteer may withdraw from the study at any time. However if the volunteer has undergone CHMI and not completed a course of appropriate antimalarial therapy then the volunteer will need to maintain contact with the Investigators for monitoring and treatment.
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved.
- There is no direct benefit from participating.
- The volunteer's GP will be contacted to corroborate their medical history and confirm that the volunteer is eligible to take part in the study. Volunteers will only be enrolled in the study if written information regarding the volunteer's medical history is obtained from the GP.
- The volunteer will be registered on the TOPS database (The Overvolunteering Prevention System; www.tops.org.uk).
- Blood samples will be taken and any leftover samples stored indefinitely for use in other, ethically approved research.

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate.

9.1.2 Informed consent questionnaire

If they do decide to participate, volunteers will be asked to complete a questionnaire testing their understanding of the trial [78]. This helps to ensure that individuals understand the trial sufficiently to give informed consent. Volunteers who answer all questions in the questionnaire correctly, will be asked to sign the consent form, a photocopy of this signed consent form will be given to them to take away and the original signed consent form will be stored in the CRF. The form will also be signed and dated by the Investigator. Volunteers 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 quiz correctly at the second attempt they may then complete the consent form and be screened for the trial. Failure to answer all the questions correctly at the second attempt will exclude them from participation.

9.2 Inclusion and exclusion criteria

9.2.1 Inclusion criteria

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

- Healthy adult aged 18 to 45 years.
- Red blood cells positive for the Duffy antigen/chemokine receptor (DARC).
- Normal serum levels of Glucose-6-phosphate dehydrogenase (G6PD).
- Negative haemoglobinopathy screen
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Willing to allow the Investigators to discuss the volunteer's medical history with their General Practitioner.
- Women only: Must practice continuous highly effective contraception* for the duration of the study
- Agreement to permanently refrain from blood donation
- Written informed consent to participate in the trial.
- Reachable (24/7) by mobile phone during the period between CHMI and completion of all antimalarial treatment.
- Willing to take a curative anti-malarial regimen following CHMI.
- Willing to reside in Oxford for the post-challenge period, until antimalarials have been completed.
- Answer all questions on the informed consent quiz correctly at first or second attempt

* Female volunteers are required to use a highly effective form of contraception during the course of the study as malaria challenge could pose a serious risk to both maternal health and the unborn foetus.

Highly effective forms of contraception for female volunteers include:

- Hormonal methods, as listed below (an additional form of contraception will be required where artemether/lumefantrine is used as the anti-malarial treatment post CHMI, as this can interfere with the efficacy of hormonal contraception):
    - 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 (progesterone only

hormonal contraception where inhibition of ovulation is not the primary mode of action is not considered highly effective)

- 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. However, where artemether/lumefantrine (Riamet) is used as the anti-malarial treatment post-diagnosis following CHMI, female participants using hormonal contraceptives must agree to using an additional method of contraception (e.g. barrier methods) from the time of commencing the artemether/lumefantrine (Riamet) drug course until the next menstrual period. This is to minimise any risk of possible reduced effectiveness of hormonal contraceptives during concomitant antimalarial therapy, due to the weak induction of cytochrome P450 enzymes resulting from treatment with artemether.

9.2.2 Exclusion criteria

The volunteer may not enter the study if any of the following apply:

- History of clinical malaria (any species).
- Travel to a clearly malaria endemic locality during the study period or within the preceding six months.
- Use of systemic antibiotics with known antimalarial 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
- Weight less than 50kg, as measured at the screening visit
- Receipt of immunoglobulins within the three months prior to planned administration of the vaccine candidate.
- Receipt of blood products (e.g., blood transfusion) at any time in the past.
- Peripheral venous access unlikely to allow twice daily blood testing (as determined by the Investigator).
- Receipt of an investigational product in the 30 days preceding enrolment, or planned receipt during the study period.
- Receipt of any vaccine in the 30 days preceding enrolment, or planned receipt of any other vaccine within 30 days preceding or following each study vaccination, with the exception of licensed COVID-19 vaccines, which should not be received between 14 days before to 7 days after any study vaccination
- Planned receipt of a COVID-19 vaccine between 2 weeks before the day of CHMI until completion of antimalarial treatment
- Concurrent involvement in another clinical trial or planned involvement during the study period
- Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data or the *P. vivax* parasite as assessed by the Investigator.
- 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).
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine e.g. egg products, Kathon
- History of allergic disease or reactions likely to be exacerbated by malaria infection.
- History of clinically significant contact dermatitis
- Any history of anaphylaxis in reaction to vaccinations
- Pregnancy, lactation or intention to become pregnant during the study.
- Use of medications known to cause prolongation of the QT interval ***and*** existing contraindication to the use of Malarone.
- Use of medications known to have a potentially clinically significant interaction with Riamet ***and*** Malarone.
- 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.
- Contraindications to the use of both of the proposed anti-malarial medications; Riamet Malarone.
- 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 abuse as defined by an alcohol intake of greater than 25 standard UK units every week.
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Hepatitis B surface antigen (HBsAg) detected in serum.
- Seropositive for hepatitis C virus (antibodies to HCV) at screening or (***unless*** has taken part in a prior hepatitis C vaccine study with confirmed negative HCV antibodies prior to participation in that study, and negative HCV RNA PCR at screening for this study).
- Positive family history in both 1st AND 2nd degree relatives < 50 years old for cardiac disease.
- Volunteers unable to be closely followed for social, geographic or psychological reasons.
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination. In the event of abnormal test results, confirmatory repeat tests will be requested. Procedures for identifying laboratory values meeting exclusion criteria are shown in SOP VC027.
- Any other significant disease, disorder, or finding 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 contact the volunteer's GP to confirm medical history and safety to participate

9.2.3 Prevention of 'Over Volunteering'

Volunteers will be excluded from the study if they are concurrently involved in another trial. In order to check this, volunteers will be asked to provide their National Insurance or Passport number (if they are not entitled to a NI number) and will be registered on a national database of participants in clinical trials (***www.tops.org.uk***).

9.2.4 Vaccination and re-vaccination exclusion criteria

The following adverse events constitute contraindications to administration of vaccine at that point in time; if any one of these adverse events occurs at the time scheduled for vaccination,

the subject may be vaccinated at a later date, or withdrawn at the discretion of the Investigator. The subject must be followed until resolution of the event as with any adverse event:

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C/99.5°F.
- Temperature of >37.5°C (99.5°F) at the time of vaccination.
- Current COVID-19 infection, defined as ongoing symptoms with positive COVID-19 PCR swab test taken during current illness or positive COVID-19 PCR swab test within preceding 14 days without symptoms. Vaccinations will be delayed by a minimum of 2 weeks from the date of the 1st positive COVID-19 PCR swab, as long as symptoms are improving or resolved. It will be at the discretion of the Investigator to withdraw a participant if they develop severe COVID-19 disease.

The following adverse events associated with vaccine immunisation constitute absolute contraindications to further administration of vaccine. If any of these events occur during the study, the subject must be withdrawn and followed until resolution of the event, as with any adverse event:

- Anaphylactic reaction following administration of vaccine
- Pregnancy

9.2.5 Exclusion criteria on day of CHMI

The following constitute absolute contraindications to CHMI:

- Acute disease, defined as moderate or severe illness with or without fever
- Current COVID-19 infection, defined as ongoing symptoms with positive COVID-19 PCR swab test taken during current illness or positive COVID-19 PCR swab test within preceding 14 days without symptoms.
- Pregnancy

9.2.6 Concomitant medications

As set out by the exclusion criteria, volunteers may not enter the study if they have received or there is planned receipt of any other vaccine within 30 days of each study vaccination, with the exception of licensed COVID-19 vaccines, which should not be received between 14 days before to 7 days after any study vaccinations. In addition volunteers who are due to receive a COVID-19 vaccine between 2 weeks before the day of CHMI until expected completion of antimalarial treatment (around 2 to 3 weeks after day of challenge based on experience in previous *P. vivax* CHMI studies to date) will not be enrolled in this trial. If a volunteer receives an appointment for COVID-19 vaccination after they have undergone malaria challenge but before reaching malaria diagnosis criteria, they will be advised to delay their COVID-19 vaccination until after reaching malaria diagnostic criteria and completion of antimalarial treatment. If a participant does not wish to delay their COVID-19 vaccination, they will be treated with antimalarial treatment at that timepoint and withdrawn from the study but will be encouraged to complete follow-up for safety.

Volunteers may also not enter the study if they have any investigational medicinal product within the 30 days prior to enrolment, or have received or plan to receive any immunosuppressant medication 6 months prior to enrolment or at any time during the study period (inhaled and topical steroids are permitted). Volunteers will also be excluded from partaking in CHMI, if there is any concomitant use of systemic antibiotics with known anti-malarial activity or anti-malarial medication within 30 days of CHMI, however, follow-up will continue until approximately 6 months (C+96), according to the schedule as long as valid informed consent remains in place.

Following CHMI, should a volunteer require treatment with systemic antibiotics at any time prior to reaching criteria for diagnosis, (as described in Section 11.4), the volunteer will be withdrawn from the study and treatment with an appropriate, curative course of anti-malarial therapy will be commenced immediately. Follow-up for safety will be continued until 96 days after challenge (C+96) in all cases except where there is complete withdrawal of consent.

9.3 Withdrawal of volunteers

In accordance with the principles of the most recent revision of the Declaration of Helsinki (2013) and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. In addition the volunteer may withdraw/be withdrawn from further study procedures at any time in the interests of the volunteer's health and well-being, or for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation.
- Volunteer non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

The Local Safety Monitor (LSM) may recommend withdrawal of volunteers. The reason for withdrawal from further study procedures will be recorded in the CRF. If a volunteer withdraws after having completed a course of antimalarials, as much continued safety data collection as possible, including procedures such as safety bloods, will be continued, with agreement of the volunteer. For all AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

Any volunteer who fails to attend for two or more follow-up visits will be deemed to have withdrawn from the study. If a volunteer withdraws from the study after CHMI but before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of anti-malarial therapy must be completed. The importance of this will be emphasised to volunteers at screening. If a volunteer refuses to take anti-malarial therapy after malaria diagnosis, a rapid assessment of mental state and capacity will be undertaken, with the involvement of NHS psychiatric and infectious diseases services. If necessary the volunteer may be detained under section 4 of the UK Mental Health Act until this assessment can be carried out.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Similarly, all data collected up to the point of withdrawal will be stored, unless they specifically request for it to be destroyed. Volunteers are free to request that their blood samples be destroyed anytime during or after the study.

In all cases of subject withdrawal, excepting those of complete consent withdrawal, safety data collection will continue until 90 days after challenge (C+90), if subjects have undergone CHMI.

9.4 Pregnancy

Should a volunteer become pregnant during the trial, she will be treated with antimalarials immediately and will be withdrawn from the study. We will not routinely perform venepuncture on such volunteers, other than blood films to check that the parasitaemia has been cleared by the antimalarials. Additionally, if a volunteer who had been negative for CMV IgG antibodies at

screening should become pregnant during the study, given the theoretical risk of CMV infection from exposure to the inoculum, venepuncture would also be performed to check CMV serostatus. With the volunteer's permission she shall be followed up until pregnancy outcome. The management of any volunteer found to be pregnant at any time after challenge up to the point of malaria treatment will be discussed with the on-call infectious diseases consultant at the Oxford University Hospitals NHS Foundation Trust, including advice on antimalarial drug choice.

Should a volunteer become pregnant after receiving antimalarial treatment (but prior to the end of the study), they shall be withdrawn from the study as soon as we have confirmed that their parasitaemia has cleared. With the volunteer's permission, she shall then be followed up until pregnancy outcome.

10 CONTROLLED BLOOD-STAGE MALARIA INFECTION INOCULUM

10.1 Preparation of the inoculum

Thawing and washing of the inoculum will be done with commercial solutions for human use and with disposable syringes and needles according to standard operating procedures used in previous studies at Oxford [108, 109]. Work will be carried out in the derogated category III laboratory at the Jenner Institute, Old Road Campus Research Building (ORCRB) or New Biochemistry Building. Sample manipulations will be performed within a safety cabinet that has been fumigated, sterilised and dedicated for this purpose.

All procedures relating to thawing of the blood inoculum and preparation of the syringes, will be performed in accordance with local standard operating procedures.

10.2 Administration of the inoculum

The inoculation will take place in Oxford at the CCVTM and according to local Standard Operating Procedure. In brief, the inoculum will be administered by intravenous injection into an indwelling intravenous cannula. Parasitised red blood cells will be reconstituted in 0.9% saline, to a total volume of 5mL. The reconstituted inoculum will be injected via an indwelling cannula, followed by a saline flush. Subjects will be observed for a minimum of one hour before discharge. All volunteers will receive the inoculum within a maximum of 4 hours of removal from frozen storage.

11 TREATMENT OF TRIAL VOLUNTEERS

This section describes the clinical procedures for evaluating study participants and follow-up after CHMI.

11.1 Trial sites

Volunteers will be recruited and undergo all visits at the CCVTM, Oxford, this is inclusive of screening, vaccinations and follow-up visits, CHMI and post-CHMI follow-up. Following CHMI until completion of anti-malarial therapy, all volunteers will reside in Oxford and the surrounding areas. After completion of treatment, volunteers may reside outside the Oxford area but will attend for later follow-up visits at the CCVTM, Oxford.

11.2 Study procedures

Procedures will be performed at the time points indicated in the schedule of procedures (tables 7-15). Additional procedures or laboratory tests may be performed, at the discretion of the Investigators if clinically necessary (e.g. urine microscopy in the event of positive urinalysis).

11.2.1 Observations

Pulse, blood pressure and temperature will be measured at the time points indicated in the schedule of procedures (Tables 7-15). Weight and height will be measured at screening and weight re-measured at the C-2 visit.

11.2.2 Blood tests

Blood will be drawn at the time points indicated in the schedule of procedures (Tables 7-15) and the following laboratory assays performed:

At Oxford University Hospitals NHS Foundation Trust, using NHS standard procedures

- **Haematology;** Full blood count, to include reticulocyte count at C-2 visit. Duffy antigen/chemokine receptor (DARC) gene positivity (either or both Fya/Fyb positive), and glucose-6-phosphate dehydrogenase (G6PD) levels and a haemoglobinopathy screen will be performed at screening only.
- **Biochemistry;** Sodium, potassium, urea, creatinine, albumin, liver function tests, C reactive protein (CRP), magnesium and calcium, however magnesium and calcium will only be measured at screening. Serum will also be tested for beta-human chorionic gonadotrophin (β-HCG) at C-2, C+7, C+14, C+21 and C+28/day of malaria diagnosis. C-reactive protein (CRP) will be measured on days 0, 1, 3, 7, 56, 57, 59 and 63 as an exploratory measure only (CRP results will not be included in safety analysis).
- **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies, CMV antibodies and EBV antibodies will be performed at screening and at day 96 post CHMI (C+96). EBV and CMV serology will only be repeated at day 96 post CHMI if they were negative at screening
- **Immunology;** Human Leukocyte Antigen (HLA) typing at enrolment (D0).

At the University of Oxford research laboratories (at the Jenner Institute and Department of Biochemistry)

- **Diagnostic Tests:** PCR for *P. vivax* DNA and gametocytes.
- **Immunology:** Immunological responses to primary *P. vivax* infection will be assessed by a variety of established and exploratory assays. This may include:
    - Antibodies and B cell responses to the *P. vivax* Duffy-binding protein (PvDBP) – total IgG, isotypes and avidity;
    - T cell responses to PvDBP by *ex-vivo* ELISpot and flow cytometry assays;
    - Functional antibody assays against parasites *in vitro*;

    - Antibody responses to whole parasites and other *P. vivax* antigens including red blood cell invasion ligands as well as the use of arrays;
    - Serum cytokine analysis.
    - B cells, plasma and/or serum may be analysed and used to produce human monoclonal antibodies against *P. vivax* malaria.
- **Genetic tests:** DNA analysis of genetic polymorphisms potentially relevant to immunological responses and gene expression studies of the host and the parasite amongst others may be performed at the discretion of the Investigators.
- **COVID-19 serology:** Antibody to SARS-COV-2 will be measured retrospectively at screening, re-screening and at the final study visit following the final CHMI for each participant (+/- at interim time points) for restrospective exploratory analysis of any association between vaccine immunogenicity and COVID-19 serostatus
- Samples may be sent to collaborating laboratories for other immunological assays or studies of malaria.
- Samples may also be sent to collaborating laboratories within and outside the UK for immunomonitoring and/or harmonisation of key immunological assays and/or studies of malaria.

Immunological assays will be conducted according to the procedures established in the test laboratories. Specific details of which immunological tests will be performed at each time-point are detailed in the separate immuno-monitoring plan – this will also clearly state which assays are conducted to meet the trial's specified objectives and which are exploratory. With the volunteers' 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 analysis of human malaria infection. This may include human DNA and RNA analysis. Volunteers can, however, request for their remaining blood samples to be destroyed at any time.

11.2.3 Urinalysis

Urine will be tested for the presence of clinically significant proteinuria, glycosuria or haematuria (as defined in Appendix A) at the screening and re-screening visits. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening, immediately prior to each vaccination and on the day of diagnosis, immediately prior to initiation of treatment.

11.2.4 Electrocardiogram

An electrocardiogram will be performed at the initial screening visit for detection of any conduction abnormalities, including long QT syndrome.

11.2.5 Vaccinations

Before each vaccination, the on-going eligibility of the volunteer will be reviewed. The vaccine will be administered as described above in section 6.4. The injection site will be covered with a sterile dressing and the volunteer will stay in the local trial site for observation for 1 hour, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes after vaccination (+/- 10 minutes).

An oral thermometer and tape measure will be given to each volunteer, with instructions on use, along with the emergency 24 hour telephone number to contact the on-call study physician if needed.

Although there is extensive safety data for the use of Matrix M1 in humans and this PvDBPII protein has been shown to be safe when administered with adjuvant GLA-SE in a phase 1a trial [29], this will be the first human trial of the combination of PvDBPII and Matrix M1.

Because of this, in the initial round of vaccination with PvDBPII-Matrix M1, the first volunteer will be enrolled and vaccinated alone and reviewed on the following day. Providing there are no safety concerns at the day 1 review, or recorded in the diary over the next 24 hours, another two volunteers may be immunised, a minimum of 2 days after the first volunteer and at least one hour apart. After a minimum of 7 days following the enrollment of the second and third volunteers, and following completion of day 7 clinic visits for all three volunteers, an internal review of safety data for the first three volunteers will be conducted. Provided there are no safety concerns at internal safety review, and pending CI approval, the remaining volunteers in Group 1 may be enrolled and vaccinated.

The second, third and fourth doses (in Group 3 only) of the PvDBPII-Matrix M1 vaccine will be administered to all volunteers on the same day, or within the specified time windows (Table 6).

11.2.6 Management of post-vaccination fevers

Post-vaccination fevers may cause diagnostic uncertainty and concurrent COVID-19 infection could be a possibility. Results from this trial to date show that post-vaccination fever does occur in a proportion of participants, following administration of the PvDBPII-Matrix M1 vaccine. After the first set of vaccinations, none of the 12 participants developed a fever; however following the 2nd set of vaccinations, 3 out of 11 participants became febrile and therefore fevers will be expected to occur in some participants following their 3rd vaccination.

If a participant develops a fever within the first 24 hours after vaccination and no other symptoms associated with COVID-19 disease (new onset cough, anosmia or ageusia), then post-vaccination fever is most likely. Participant will be advised to self-isolate until they become afebrile for 24 hours. Participants will be requested to attend their day 1 post vaccination study visit to allow collection of study samples including safety blood tests. This study visit will be conducted in a side room with study staff wearing appropriate PPE and participants will be asked to attend the visit by means other than public transport.

If a participant develops a fever post vaccination, which is associated with either new onset cough or anosmia or ageusia, they will be advised to self-isolate as per Public Health England (PHE) guidance and directed to local community based COVID-19 testing. If participants remain febrile more than 48 hours post vaccination, they will be advised to continue self-isolation and directed to local community based COVID-19 testing. Whilst awaiting their COVID-19 PCR test results, participants will be advised not to attend the clinic for study visits.

If a participant tests positive for COVID-19 or whilst awaiting their COVID-19 test result, they will be advised to continue self-isolating as per PHE guidance. Any study visits which are due whilst a participant is self-isolating, should be rescheduled to occur when self-isolation ends, if this falls within the visit window. If a participant remains in self-isolation during the period of the visit window, then the study visit will be conducted by telephone and no blood tests will be taken. Study visit windows have been increased in order to accommodate these scenarios.

If a participant is self-isolating for a prolonged period post-vaccination (for example because they are a household contact of a suspected or confirmed COVID-19 case) and hence would miss all three initial post vaccination visits, then it will be at the Investigator's discretion to conduct these as physical visits at CCVTM to allow full assessment including blood tests. These study visits would be conducted in a side room with study staff wearing level 1 Personal Protective Equipment (PPE).

If a participant tests negative for COVID-19, they can stop self-isolating and continue with study visits as planned.

11.2.7 Diary card

Following each vaccination, volunteers will be asked to complete a diary card which may be in paper or electronic form. Volunteers will be asked about foreseeable local and systemic AE's for 7 days (solicited AEs). After this, volunteers will be asked to record any AE daily, for 28 days (unsolicited AEs). Diary cards will be reviewed with volunteers at each post-vaccination clinic visit.

11.2.8 SARS-COV-2 PCR swab

Whilst the COVID-19 pandemic is ongoing, a combined nasopharyngeal and throat swab will be tested for SARS-COV-2 by PCR two days before malaria challenge (C-2) in all volunteers. Asymptomatic COVID-19 infection will be a contra-indication to proceeding to CHMI. All participants who undergo CHMI will also have a COVID-19 swab taken on the day of malaria diagnosis when malaria treatment is commenced. Any participant who develops symptoms, which may be consistent with COVID-19 disease after undergoing malaria challenge and prior to commencing malaria treatment, may also undergo testing for COVID-19 by PCR swab. Swabs from symptomatic participants will be taken by the study team in-house but in a physically separate location from the main clinic area with separate side access, with full infection control precautions (as per the PPE SOP).

If a participant develops symptoms consistent with COVID-19 after completing malaria treatment, they will be referred to local community testing for COVID-19.

11.3 Study visits

All clinical reviews and procedures will be undertaken by one of the clinical team. The procedures to be included in each visit are documented in Tables 7-15. Each review is assigned a time point and a window period within which the review will be conducted.

11.3.1 Screening visits

Where possible, and where consent to contact by telephone has been provided by email, individuals may be phoned by a member of the clinical team to discuss the study, prior to the screening visit. This pre-screening telephone call will provide an opportunity for interested individuals to discuss the study, eligibility criteria and study requirements. Where a pre-screening call takes place, if individuals continue to express an interest in taking part in the trial following the pre-screening call, a screening visit will be scheduled.

Screening visits may take place up to 90 days prior to enrolment. Informed consent will be gained at screening. If consent is given, the screening procedures indicated in the schedule of procedures (Table 7) will be undertaken, including testing for Duffy antigen/chemokine receptor (DARC) positivity, G6PD levels and haemoglobinopathy screen.

The subject's general practitioner will be contacted with the written permission of the subject after screening to ascertain any significant medical history and as notification that the subject has volunteered for the study. During the screening the volunteers will be asked to provide their National Insurance or passport number so that this can be entered on to a national database which helps prevent volunteers from participating in more than one clinical trial simultaneously or over-volunteering for clinical trials (www.tops.org.uk).

Abnormal clinical findings from the medical history, physical examination, urine or blood tests at any point in the study will be assessed according to the scales in Appendix A and site specific

laboratory adverse event grading tables kept in the trial master file (ref: SOP VC027). If a test is deemed clinically significant it may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer. Exclusion of the volunteer from enrolling in the trial or withdrawal of a volunteer from the trial will be at the discretion of the Investigator.

Where more than 90 days has lapsed between the initial screening visit and date of enrolment, a re-screening visit will be conducted to ascertain if there have been any changes in the medical history, medical examination findings or urinalysis, haematology or biochemistry blood tests only. Other blood tests and ECG will not be repeated routinely. In addition, where a reply from the subject's GP has already been received, the GP will not be re-contacted unless there is any need to obtain further information where appropriate (e.g. regarding a new medical event or finding) and at the discretion of the Investigator.

Participants who are currently enrolled in Group 1 and consent to continue in the study, will attend a re-screening visit prior to their third vaccination due to the long duration of the trial halt.

Participants in Group 1 who would like to enrol into Group 3, will be re-consented prior to enrolment into Group 3. However they will not require a re-screening visit because their last visit C+96 in Group 1 will be only be about 1 month from enrolment into Group 3.

11.3.2 Enrolment and first vaccination with PvDBPII-Matrix M1

The eligibility of the volunteer will be reviewed at the end of the screening visit and again when all results from the screening visit have been considered. If eligible, a day 0 visit will be scheduled for the volunteer to receive the first dose of vaccine and be enrolled in the trial.

At the day 0 visit, any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β HCG test in female volunteers and venepuncture for immunology and safety bloods will be undertaken according to Table 7. The inclusion and exclusion criteria for the study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix M1 vaccine will be administered as described in section 6.4.

11.3.3 Reviews post-vaccination on days 1, 3, 7 and 14

On subsequent visits, the volunteers will be assessed for local and systemic adverse events, using a diary card, interim history, physical examination and blood tests at the time points indicated in the schedule of attendances (Table 7). Blood will also be taken for exploratory immunology analysis.

Volunteers will be asked to record all AEs via the diary card and upon attending clinic visit on days 1, 3 and 7, during which diary cards will be reviewed with volunteers. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). Volunteers will be asked about any new symptoms from day 7 until day 28, on a daily basis via the diary but will not be asked directly about foreseeable symptoms. Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 7.

11.3.4 2nd Vaccination with PvDBPII-Matrix M1 (day 28)

This visit will include a follow up visit for the first vaccination and administration of the second vaccine. Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β HCG test in female volunteers and venepuncture for immunology and safety bloods will be undertaken according to Table 7. The inclusion and exclusion criteria for the

study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix M1 vaccine will be administered intramuscularly in the opposite arm to that used for the first vaccination and as described in sections 6.4.

11.3.5 Reviews post-vaccination on days 29, 31, 35 and 42

The volunteers will be assessed for local and systemic adverse events, using a diary card, interim history, physical examination and blood tests at the time points indicated in the schedule of attendances (Table 7). Blood will also be taken for exploratory immunology analysis.

Volunteers will be asked to record all AEs via the diary card and upon attending clinic visit on days 29, 31 and 35, during which diary cards will be reviewed with volunteers. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). Volunteers will be asked about any new symptoms from day 35 until day 42, on a daily basis via the diary but will not be asked directly about foreseeable symptoms. Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 7. At any post-vaccination visit, where valid written, informed consent is in place, photographs may be taken for clinical comparison. Confidentiality will be maintained as described in Section 15.5.

11.3.6 3rd Vaccination with PvDBPII-Matrix M1 (day 56 or approximately day 365 for Group 1)

For Group 2, this visit will include a follow up visit for the second vaccination and administration of the third vaccine. For Group 1 the 3rd vaccination will occur between 12 to 18 months after the second vaccination depending on the duration of the trial halt.

Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β HCG test in female volunteers and venepuncture for immunology and safety bloods will be undertaken according to Table 7. The inclusion and exclusion criteria for the study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix M1 vaccine will be administered intramuscularly in the opposite arm to that used for the second vaccination and as described in sections 6.4.

11.3.7 Reviews post-vaccination on days 57, 59, 63, and 70 for Group 2 (or 1, 3, 7 and 14 days post 3rd vaccination for Group 1)

Volunteers will be assessed for local and systemic adverse events, using a diary card, interim history, physical examination and blood tests at the time points indicated in the schedule of attendances (Table 7). Blood will also be taken for exploratory immunology analysis.

Volunteers will be asked to record all AEs via the diary card and at visits on days 57, 59 and 63 for Group 2 (or 1, 3 and 7 days post 3rd vaccination for Group 1), during which diary cards will be reviewed with volunteers. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). Volunteers will be asked to continue to record any new symptoms from day 70 until day 84 for Group 2 (or from 14 to 28 days after the 3rd vaccination for Group 1), on a daily basis via the diary but will not be asked directly about foreseeable symptoms. Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 7.

11.3.8 Group 3 only – 4th Vaccination with PvDBPII-Matrix M1 and reviews on 1, 3, 7 and 14 days post-vaccination

For Group 3 only the 4th vaccination will occur approximately 5 months after their third vaccination and about 1 month after the volunteers have completed their C+96 visit following

the first CHMI (as part of Group 1). Participants will be re-consented for enrolment into Group 3, if not already re-consented at a prior visit. Enrolment into Group 3 will occur at the point of the fourth vaccination.

Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β HCG test in female volunteers and venepuncture for immunology and safety bloods will be undertaken according to Table 12. The inclusion and exclusion criteria for the study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix M1 vaccine will be administered intramuscularly.

Following the 4th vaccination, volunteers will be asked to record all AEs via the diary card and upon attending clinic visit on days 1, 3 and 7 after vaccination, during which diary cards will be reviewed with volunteers. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). Volunteers will be asked about any new symptoms from day 7 until day 28 after vaccination, on a daily basis via the diary but will not be asked directly about foreseeable symptoms. Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 12.

11.3.9 Two days before CHMI (C-2 (±1))

This visit will include a follow up visit for the final vaccination, as well as assessment for eligibility for CHMI. Any new medical issues or symptoms that have arisen will be assessed. Physical observations, serum β-HCG test in female volunteers and venepuncture for immunology, malaria PCR and safety bloods will be undertaken according to Table 8 for Group 1 and Table 13 for Groups 2 and 3. A combined nasopharyngeal and throat swab for SARS-COV-2 PCR will also be taken. Results of safety bloods and the SARS-COV-2 PCR test taken at this visit must be available and reviewed prior to challenge. Any participant with a positive SARS-COV-2 PCR swab will not proceed to CHMI.

11.3.10 Day of CHMI (C0)

CHMI will be administered, according local standard operating procedure (SOP), this will include

- Interim history and examination of the injection site and any body systems felt to be necessary by the Investigator and verify continuing eligibility / contraindications.
- Baseline physical observations (respiratory rate, pulse, blood pressure and temperature)
- Intravenous access via cannulation in forearm vein, flushed with normal saline.
- For each volunteer the inoculum must be injected within 4 hours of the inoculum being thawed, followed by a further normal saline flush.
- Physical observations will be repeated at 15 minutes (+/- 5 minutes) post-administration of the inoculum and again at 1 hour (+/- 20 minutes), in order to assess for immediate adverse reactions, with regular visual observation throughout. If the volunteer should show signs or symptoms of a transfusion reaction, observations will be repeated and the volunteer will be medically reviewed by a physician.
- Volunteers will be observed for at least 60 minutes, however, this period may be extended if there are any clinical concerns.
- If there have been no symptoms or signs indicative of a transfusion reaction, the cannula will be removed after 1 hour

Before leaving CCVTM, volunteers will be informed about the unlikely possibility of a delayed transfusion reaction and will be provided with the 24 hour emergency mobile telephone number, to enable them to contact a physician in the event of concern.

A Medic-Alert type card will be issued to each volunteer. This will contain the study physician contact details and a request that the research team be contacted immediately in the event of illness/accident. Each subject will also be issued with an accurate oral thermometer. If the subject 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. In addition full contact details for each subject will be documented, including home and work addresses, home and work landline telephone numbers where available and next-of-kin address and telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the volunteers are easily contactable. Subjects must also provide the Investigators with the 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.

Volunteers must be resident in Oxford / the surrounding area until they have had a least one negative thick blood film, and (/or, if not diagnosed on the basis of blood-film) two consecutive qPCR results with substantial reduction in genome copies/mL after commencing antimalarial treatment. If volunteers do not reach malaria diagnosis, they must remain in the Oxford area until they have completed anti-malarial treatment.

Volunteers will be counselled that should they fail to return for treatment having been infected with *P. vivax* malaria they could become very unwell and potentially die. They will be informed that should they fail to attend a scheduled clinic visit post-challenge, their nominated contact, next of kin and the police may be informed and a search started.

While not frequent practice, accommodation in Oxford for the post-challenge follow-up period can be arranged on a case-by-case basis for eligible volunteers who do not reside in the Oxford area. The costs of this will be covered by the research group.

11.3.11 Days 1-6 post-CHMI (C+1 – C+6)

In the VAC069 study, three doses of inoculum were assessed for feasibility of infection. Two volunteers received the equivalent of a whole vial of infected erythrocytes, two further volunteers received a 1:5 dilution and two received a 1:20 dilution. All volunteers were completely asymptomatic in the first 6 days. Patent parasitaemia, as determined by qPCR, was first detected after day 7, in a volunteer who had received the highest dose. For the same volunteer, microscopic patency was at day 12 and diagnosis was made on the same day (C+12.5). For the remaining volunteers, qPCR became positive after day 8 and microscopic patency was no earlier than day 14. Diagnostic criteria for all remaining volunteers were reached between days 15 and 16. Therefore, a mid-range dose of 1:10 dilution is to be used in this study. qPCR parasitaemia patency is not expected until at least 7 days after challenge and so volunteers will not need to be reviewed in clinic until day 7. However, they will be phoned daily by the clinic team from day 1 to 6 to make sure they are well and contactable, and will be able to contact the study physicians on the 24 hour emergency telephone number if needed.

11.3.12 Days 7-28 post-CHMI (C+7 – C+28) – Group 1

Volunteers will be reviewed in clinic once in the morning of day 7 after challenge until day 9, then twice daily from day 10 until diagnosed, or if diagnosis criteria is not met, until day 28 post-challenge inclusive (C+10 to C+28). However, from day 21 after challenge, visits may be reduced to once daily at the discretion of the Investigator (as agreed by the Chief Investigator) e.g., if qPCR is very low or undetectable, and volunteer is completely asymptomatic (qPCR results may be unblinded for this purpose).

At each follow-up visit:

- Physical observations will be performed. Venepuncture will be performed as per schedule of attendance (Table 8, 9, 10 and 11), including blood for assessing parasite growth rate, immune response and level of gametocytaemia.
- Volunteers will be questioned as to whether they have:
    - Experienced any of the foreseeable symptoms of malaria.
    - Experienced any other symptoms.
    - Taken any medications including over the counter medications.

Full physical examination will be performed if deemed necessary by the Investigators. Subjects will be encouraged to contact one of the Investigators on the 24 hour emergency mobile telephone number if they develop symptoms of malaria or concerning AEs between the regular clinic reviews. The severity of symptoms will be assessed using grading criteria summarized in Section 12 below.

Diagnosis will be made according to criteria described in section 11.4 below. If volunteers reach day 28 post-challenge (C+28) without reaching diagnostic criteria, they shall be started on anti-malarial treatment empirically. Subjects will be reviewed in clinic approximately 24 and 48 hours after starting anti-malarial therapy as per Table 8 below.

If a volunteer is unwell and unable to attend the CCVTM for a visit they will be reviewed on the phone and visited at home by one of the Investigators if necessary. Such visits will be conducted according to SOP VC022: Lone Working in the Community.

11.3.13 Days 7-21 post-CHMI (C+7 – C+21) – Groups 2 and Group 3

Following review of the results from the first CHMI in Group 1 volunteers, the number of visits in the post challenge period has been reduced to minimise the number of bleeds and burden on volunteers. During the first CHMI, five volunteers in Group 1 continued with twice daily visits from C+10 until day of diagnosis, which occurred between C+19 to C+25 for these volunteers. One volunteer in Group 1 reduced to once a day visits from C+21 to C+25 before being diagnosed at C+27.

Review of the qPCR results and parasite multiplication rate (PMR) modelling from the CHMI in Group 1 and the corresponding VAC069 control volunteers has shown that sufficient data can be obtained for accurate PMR modelling if bleeds for qPCR continue once a day until qPCR reaches ≥1000 genome copies/ml, instead of starting twice a day visits from C+10 onwards. Once qPCR levels reach ≥1000 genome copies/ml, then visits will increase to twice a day in order to not delay diagnosis as qPCR may reach the diagnostic threshold of ≥5000 genome copies/ml with malaria symptoms within about half a day. PMR modelling is also minimally affected if, in addition to reducing the frequency of bleeds to once daily, participants are treated at C+21, instead of C+28, even if they have not reached malaria diagnostic criteria at that timepoint.

Group 2 and 3 volunteers will therefore be reviewed in clinic once a day in the mornings from day 7 after challenge until day 9. From day 10 after challenge onwards visits continue once a day until the participant's qPCR count reaches ≥1000 genome copies/ml, at which time visits will increase to twice a day until diagnosed, or if diagnosis criteria are not met, until day 21 post-challenge inclusive (C+21).

At each follow-up visit:

- Physical observations will be performed. Venepuncture will be performed as per schedule of attendance (Table 13, 14 and 15), including blood for assessing parasite growth rate, immune response and level of gametocytaemia.
- Volunteers will be questioned as to whether they have:
    - Experienced any of the foreseeable symptoms of malaria.
    - Experienced any other symptoms.

- Taken any medications including over the counter medications.

Full physical examination will be performed if deemed necessary by the Investigators. Participants will be encouraged to contact one of the Investigators on the 24 hour emergency mobile telephone number if they develop symptoms of malaria or concerning AEs between the regular clinic reviews. The severity of symptoms will be assessed using grading criteria summarized in Section 12 below.

Diagnosis will be made according to criteria described in section 11.4 below. If volunteers reach day 21 post-challenge (C+21) without meeting diagnostic criteria, they shall be started on anti-malarial treatment empirically. Subjects will be reviewed in clinic approximately 24 and 72 hours after starting anti-malarial therapy as per Table 15 below.

If a volunteer is unwell and unable to attend the CCVTM for a visit they will be reviewed on the phone by a clinician. If inperson assessment is considered necessary, the participant will be referred to appropriate NHS services.

11.4 Algorithm for initiation of treatment

It is now well established that nucleic acid amplification-based diagnostics have greater sensitivity than microscopic methods for detecting malaria infection both in clinical infection and in the *P. falciparum* challenge model. In the latter, qPCR targeting the 18S ribosome DNA target has been used as the diagnostic tool, reliably detecting parasitaemia earlier than thick film blood smear.

The qPCR assay that we have used in numerous Oxford-led CHMI *P. falciparum* challenge studies is now qualified and highly sensitive, reliably detecting parasitaemias as low as 20 parasites per mL of blood (0.02 parasites per µL), long before clinical symptoms manifest. The qPCR assay has also performed well in an international External Quality Assurance (EQA) exercise [110]

It is also important to note that other trial centres in the USA routinely use PCR as a diagnostic tool in CHMI studies. NCT020-15091 was a multi-institution, Phase I, open-label, dose-escalation trial with CHMI, designed to assess the safety, immunogenicity, and protective efficacy of PfSPZ vaccine and this used PCR as the primary diagnostic [111].

For *P. vivax*, we also use a qPCR assay targeting the pan-plasmodium 18S ribosome DNA target. The only other centre worldwide that routinely performs blood-stage challenge studies for *P. vivax* (QIMR, Australia), uses PCR as the sole diagnostic tool in CHMI studies, and our qPCR assay is based on this, utilising identical primer and probe sequences. In all other aspects, apart from the QIMR derived detection sequences and reaction mixture employed, the extraction of DNA and qPCR is identical to the existing and formally validated *P.falciparum* qPCR method employed as sole, objective, diagnostic tool in the two *P.falciparum* trials described below. This pan-plasmodium qPCR is in the process of undergoing a similar formal validation and work completed so far, demonstrates that this is similarly fit for purpose. As examples of qualification parameters that contribute to validation, 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 50-100 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 validated *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) (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, as described in Figure 2, 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.

Positive thick smear results may result from chance findings, since following blood-stage challenge, parasites may be seen on light microscopy at any time-point. Where this occurs, and premature diagnosis results, this limits the qPCR dataset available for PMR modelling. Removing this possibility, therefore, increases the data available for analysis of the primary outcome measure. In addition, use of qPCR as the sole diagnostic tool leads to greater standardisation of diagnosis across study groups, permitting improved inter-group as well as inter-trial comparison. In Oxford, we have now safely conducted two *P. falciparum* blood-stage CHMI studies, where qPCR is the sole diagnostic method (Clinicaltrials.gov identifiers NCT03906474 and NCT02927145). Analysis of the parasitaemia at diagnosis in one of these studies, VAC063B, indicates that removal of microscopy as a diagnostic measure, reduced the range over which diagnosis was made compared to two prior *P. falciparum* blood-stage CHMI studies, where microscopy was utilised (see Figure 7).

As microscopy findings are not considered within the primary efficacy analysis for this study, microscopy for VAC071 Group 1 participants (n=3) (NCT04009096) was principally utilised as an additional diagnostic tool for safety reasons. The data from the two previous *P. vivax* blood-stage CHMI studies were therefore analysed to apply the revised diagnostic criteria retrospectively, to determine if timing of diagnosis would have been altered. Using the revised criteria, the majority of diagnoses would not have been delayed, although it is estimated 36% of volunteers would have had a small delay to diagnosis. However, since clinical criteria remain integral to the diagnostic algorithm, this small delay should not result in any increased risk to volunteers, and the ability for investigators to treat any participant in the event of clinical concern, following discussion with the CI, regardless of parasitaemia, will ensure participant safety.

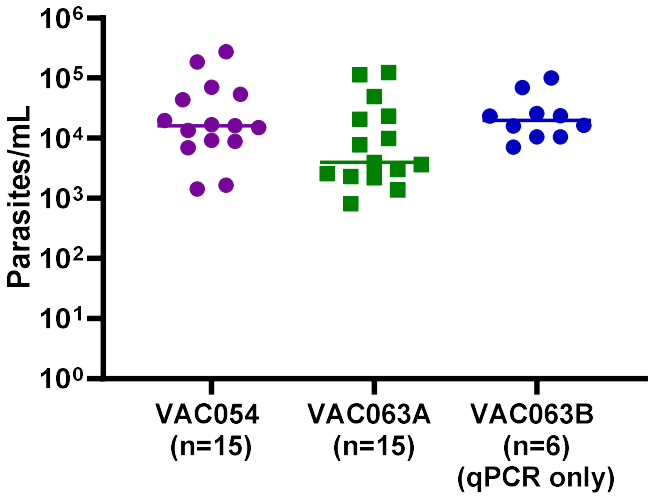

Figure 7: Parasitaemia at the diagnosis timepoint in unvaccinated control volunteers in three prior *P. falciparum* blood-stage CHMI studies.

In both the VAC054 [94] and VAC063 CHMI studies, where microscopy was used as a diagnostic measure alongside qPCR and clinical assessment, diagnosis was made over a wider range of parasitaemia compared to the VAC063B study, where thick blood smear microscopy was not performed. Diagnostic parasitaemia ranged between 1440-273,247 parasites/mL in the VAC054 study, 815- 124063 parasites/mL in VAC063A and 7137- 100946 parasites/mL in the VAC063B CHMI. Individual and median data points are shown.

On this basis diagnostic criteria in VAC079 will be based on symptoms in-keeping with malarial infection results and real-time quantitative polymerase chain reaction (qPCR) only, i.e. thick film microscopy will not be used as a diagnostic measure, as summarised in the algorithm in Figure 8.

Symptoms or signs that will be considered as indicative of symptomatic malarial infection will be fever >37.8^{o}C, rigors or other severe symptoms or signs related to malarial infection, including, but not limited to, subjective feverishness, sweats, headache, myalgia, arthralgia, nausea or vomiting.

Asexual blood-stage parasitaemia will be measured by qPCR for the pan-plasmodium 18S DNA gene. In the presence of symptoms or signs indicative of malarial infection, ≥5,000 genome copies/mL will be considered diagnostic but a PCR result ≥10,000 copies/mL will be considered positive, regardless of symptoms. All Investigators (except the Principal Laboratory Investigator) will be blinded to the results. Blinding of PCR operators to the vaccination status of a volunteer (as opposed to an infectivity control in the parallel VAC069 study) will be maintained by assigning a new unique identifier to the volunteer for PCR samples from the C-2 visit. If, as judged by the investigator, a volunteer develops symptoms in-keeping with malaria, the volunteer will be discussed with the Chief Investigator. If deemed appropriate by the Chief Investigator, the qPCR results for this volunteer will be unblinded by the Principal Laboratory Investigator and the volunteer treated only if any available qPCR result is ≥5000 genome copies/mL. At the point that a volunteer has a qPCR result exceeding 10,000 genome copies/mL, the Principal Laboratory Investigator will unblind the Chief Investigator to the result and the volunteer will be treated, regardless of the presence of symptoms.

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

The Investigators are able to treat any volunteer for malaria regardless of the qPCR result if they are clinically concerned (and have discussed the case with the Chief Investigator), or a volunteer wishes to withdraw from the study. If necessary, volunteers can be discussed with the LSM and/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.

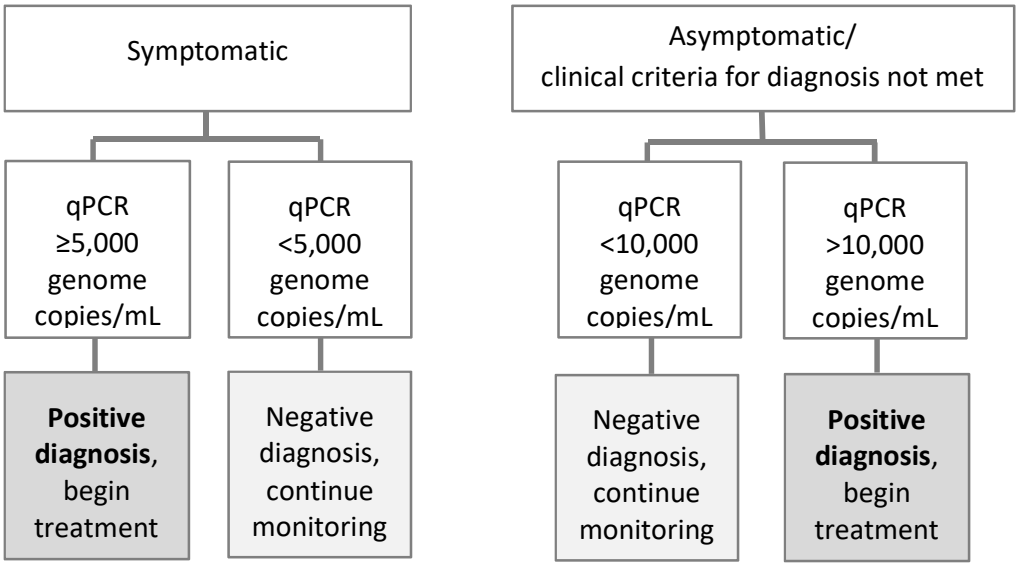

Figure 8: ***Algorithm for diagnosis and initiation of treatment.*** *N.B. If day 28 after challenge for Group 1, or day 21 for Groups 2 and 3, is reached without meeting any diagnostic criteria, treatment will be initiated regardless of qPCR results, i.e. even if parasitaemia is undetectable.*

11.4.1 Participants with fever post malaria challenge and COVID-19 testing

In the first week following CHMI, if a participant becomes unwell with a febrile illness, it is highly unlikely that the cause of this is malaria. If a participant develops a fever between days C+1 to C+6 and their symptoms are consistent with possible COVID-19 disease (fever or new onset persistent cough or anosmia or ageusia) the participant will be advised to self-isolate as per Public Health England (PHE) guidelines and directed to local community based COVID-19 testing. If the SARS-COV-2 PCR test is negative, the participant can stop self-isolation and continue with study visits as per protocol.

From C+7 onwards, if a participant develops a fever of ≥37.8°C or other symptoms consistent with possible COVID-19 disease, they will be advised to inform the study team before attending their clinic visit and commence self-isolation as per PHE guidelines. As long as the participants' symptoms are judged by the Investigator as not significant enough to require referral to secondary care, they will be advised to attend the study clinic where their visit will be conducted in a separately-accessed isolated clinic room with study staff wearing appropriate PPE. If a participant fulfils COVID-19 testing criteria, a SARS-COV-2 PCR swab from the nasopharynx and throat will be taken by the study team in addition to the study specified malaria blood tests.

If a participant has a persistent fever or study clinicians are clinically concerned for COVID-19 disease, then a SARS-COV-2 PCR swab can be repeated at the discretion of the Investigator, if the first SARS-COV-2 PCR was negative.

If a participant is self-isolating because of symptoms of possible COVID-19 disease and they are unable to travel to the clinic safely, then a home visit may be conducted by the study team in lieu of a clinic visit to allow collection of safety and malaria blood tests.

If following malaria challenge and prior to malaria diagnosis and treatment, a participant is found to have a positive SARS-COV-2 PCR swab from any source, they will be commenced on malaria treatment, irrespective of the severity of COVID-19 disease and irrespective of malaria symptoms or malaria qPCR result at that time point.

All volunteers will undergo a combined nasopharyngeal and throat swab for SARS-COV-2 PCR on the day of malaria diagnosis when malaria treatment is commenced, unless they have had a negative COVID-19 PCR swab within the preceding 24 hours. The reasoning for this is that fever after commencement of malaria treatment is common and if a participant develops a fever after starting malaria treatment, a negative COVID-19 swab will allow exclusion of concurrent COVID-19 disease and negate the need for self-isolation and household isolation.

11.5 Malaria management

Volunteers will be treated with a standard oral course of either artemether-lumefantrine (Riamet) or atavoquone/proguanil (Malarone). Both are recommended treatment of *P. vivax* infection in the UK, although use for non-falciparum malaria is an unlicensed use for both medications. The decision on which medication will be used, will be at the Investigator's discretion and depends on factors including if there are any contraindications to either medication.

If a volunteer withdraws/is withdrawn from the study after administration of CHMI but before reaching the criteria for malaria treatment, then a complete, appropriate, curative course of anti-malarial therapy must be completed. The importance of this will be emphasised to volunteers at screening.

11.5.1 Malaria management – Riamet

Artemisinin-based combination therapies (ACTs) containing mefloquine, piperaquine or lumefantrine are now the recommended treatment for *P. vivax* in areas where there might be chloroquine resistance (WHO 2015 guidelines). Although chloroquine remains effective in most recent studies in Thailand, there are some signs that low level chloroquine resistance may be becoming established along the Thai-Myanmar border.

Riamet® is a combination drug consisting of 20mg artemether and 120mg lumefantrine per tablet. The treatment regime will consist of 6 doses of total 80mg artemether/480mg lumefantrine (4 tablets) – to clear the blood-stage infection. 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, and volunteers will record the time that intervening doses were taken at home in a medication diary.

Prior to starting Riamet®, volunteers will be screened for drug interactions and contraindications. This includes checking for pregnancy by urine pregnancy test and serum β-hCG test in female volunteers and prolonged QT (on pre-challenge ECG). Volunteers will be reminded of the potential side effects and given the patient information sheet for Riamet® and a card outlining when their doses of Riamet should be taken. Volunteers will be advised to avoid grapefruit juice. Tablets should be taken together with a fatty meal (a light snack will provided when doses are observed in clinic). At least two doses for each volunteer will be directly observed in clinic.

11.5.2 Malaria management – Malarone

Malarone (see SmPc for Malarone) is a combination drug consisting of proguanil hydrochloride and atovaquone. A treatment course of Malarone consists of 4 'standard tablets' of Malarone (proguanil hydrochloride 100 mg, atovaquone 250 mg) once daily, orally for 3 days. The first two doses of Malarone will be directly observed in clinic. Prior to starting Malarone, volunteers will be screened for drug interactions and contraindications (including a serum β-hCG test in female volunteers). Volunteers will be reminded of the potential side effects of Malarone and given the patient information sheet for Malarone.

11.5.3 Malaria management – supportive medications

Provided there are no contraindications, volunteers will be provided with a course of paracetamol (1 g orally up to four 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. Volunteers will be given the patient information sheet for these medications and advised how frequently they can take doses. Volunteers will be issued with a medication diary card on which they will be asked to document all doses of medications taken post-CHMI.

All medications used in the trial will be handled and dispensed according to SOP VC021: Handling, Storage and Dispensing of Non-IMP Medication.

11.5.4 Follow-up after commencing malaria treatment

For Group 1 volunteers, at each visit, 24 hours and 48 hours after initiation of treatment, procedures will be performed according to Table 11, including physical observations, assessment of AEs and venepuncture for blood tests including measurement of malaria qPCR. Once daily malaria qPCR blood tests will continue until the volunteer has two consecutive qPCR readings with substantial reduction in genome copies per mL.

For Group 2 and 3 volunteers post treatment visits will occur at 24 hours and 72 hours after initiation of treatment. During the first CHMI, a number of volunteers in Group 1 attended for an additional visit on T+3 as the malaria parasite count at T+2 was still positive for some participants. All T+3 malaria qPCRs results were significantly reduced. In order to minimise the number of visits, the T+2 visit has been changed to T+3. Procedures will be performed according to Table 15, including physical observations, assessment of AEs and venepuncture for blood tests including measurement of malaria qPCR. Malaria qPCR blood tests can be repeated if qPCR readings are not substantially reduced by T+3.

11.5.5 Criteria for inpatient transfer to the NHS

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 antimalarial 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 subject's home circumstances.
- Any other significant finding which the Investigators feels warrant inpatient admission - this includes symptoms or signs of suspected worsening COVID-19 disease e.g. respiratory failure, in the unlikely event of a participant testing PCR positive for COVID-19 during the post-challenge period.

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

11.6 Safety measures for conduct of CHMI

Volunteer safety is of paramount importance. The following measures are in place to safeguard volunteer safety:

- Volunteers will only be enrolled in the study if both Investigators and the volunteers' GP feel this is appropriate.
- Volunteers' 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.
- If the subject 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.
- Before challenge, 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 volunteers 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.
- On the day of challenge volunteers will be provided with a medic alert card containing contact details for the study team and brief details of the study including the optimal treatment for *P. vivax*.
- Volunteers 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.
- If necessary, the study team will visit volunteers in their own homes if they are unable to attend clinic for review.
- At least two doses of Riamet® for each volunteer will be observed by the study team. (For volunteers taking Malarone, two out of three doses will be observed).
- Volunteers 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 Oxford or the immediate surrounding area for the duration of the intensive follow-up schedule (days 0-30 post-challenge). They will be informed that should they fail to attend a scheduled clinic visit post-challenge, their nominated contact, next of kin and the police may be informed and a search started.

11.6.1 Measures to be taken if a volunteer goes missing post-CHMI

In the unlikely event that a volunteer should (a) fail to attend for a scheduled clinical visit or (b) be un-contactable by telephone after challenge and before completion of an appropriate course of anti-malarial therapy, the following stakeholders will be informed;

- All Investigators.
- The volunteer's nominated contact and next of kin.
- The trial Sponsor.
- The local safety committee.
- The ethics committee(s).
- Relevant hospital trust R&D departments.
- The local police department.

- Local Accident and Emergency departments.

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

11.7 Follow-up post-treatment

11.7.1 Six days after initiation of treatment (T+6)

Physical observations will be performed and AEs assessed. Venepuncture will be performed (Table 11 for Group 1, Table 15 for Groups 2 and 3). Where applicable, medication diary and malaria symptom diary cards will be collected from volunteers.

11.7.2 Nine days after initiation of treatment (T+9)

Volunteers will be phoned at 9 days post day of diagnosis visit (treatment initiation) (as per Table 11 for Group 1, Table 15 for Groups 2 and 3). Participants will be questioned as to whether they have;

- Experienced any of the foreseeable symptoms of malaria.
- Experienced any other symptoms.
- Taken any medications including over the counter medications.

11.7.3 Day 56 post CHMI (C+56)

Physical observations will be performed and AEs assessed. Venepuncture will be performed (Table 11 for Group 1, Table 15 for Groups 2 and 3).

11.7.4 Day 96 post CHMI (C+96)

Physical observations will be performed and AEs assessed. Venepuncture will be performed (Table 11 for Group 1, Table 15 for Groups 2 and 3).

11.7.5 Day 276 post CHMI (C+276)

Physical observations will be performed and AEs assessed. Venepuncture will be performed (Table 11 for Group 1, Table 15 for Groups 2 and 3).

	S	V1					V2					V3				
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Timeline (days) Group 1		0	1	3	7	14	28	29	31	35	42	approx 365	1 day post 3rd vacc	3 days post 3rd vacc	7 days post 3rd vacc	14 days post 3rd vacc
Timeline (days) Group 2		0	1	3	7	14	28	29	31	35	42	56	57	59	63	70
Window (days)	-90	±1	±1	-1/+2	-2/+4	-3/+5	-7/+14	±1	-1/+2	-2/+4	-3/+5	-7/+14	±1	-1/+2	-2/+4	-3/+5
Inclusion/Exclusion criteria	X	X					X					X				
Informed consent	X	(X)					(X)					(X)				
ICQ	X															
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/examination	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
ECG	X															
Urinalysis	X															
Urine β-hCG**	X	X					X					X				
Review contraindications		X					X					X				
Review AEs & medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaccination		X					X					X				
Diary card issued		X					X					X				
Diary card collected							X					X				
Immunology (mL)		80	20	20	40	40	60	20	20	40	40@	80	20	20	50	80
RNA preservation (mL)		3	3				3	3				3	3			
Haematology (mL)#	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Biochemistry (mL) ##	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Serum βhCG (mL)**																
DARC (mL)	2															
HLA typing (mL)		4														

HIV, HBV, HCV, EBV, CMV serology (mL)	5															
Group 1 - Total Blood Volume (mL)^	14	92	28	25	45	45	68	28	25	45	5	88	28	25	55	85
Group 2 - Total Blood Volume (mL)^	14	92	28	25	45	45	68	28	25	45	45	88	28	25	55	85
Group 1 - Cumulative blood volume (mL)^	14	106	134	159	204	249	317	345	370	415	420	508	536	561	616	701
Group 2 - Cumulative blood volume (mL)^	14	106	134	159	204	249	317	345	370	415	460	548	576	601	656	741

Table 7: Group 1 and 2 - Schedule of events pre-CHMI

S** = screening visit; **V1, V2 and V3** = 1st, 2nd and 3rd vaccinations with PvDBPII/Matrix M1; **ICQ** = Informed Consent questionnaire**; (X)** = If considered necessary, emphasising any acute complaints. ** Physical observations will include blood pressure, pulse and temperature, plus height and weight at screening only. ****** For females only. **#** Haematology will include full blood count plus G6PD level and haemoglobinopathy screen at screening visit only. **##** Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests; CRP on days 0, 1, 3, 7, 56, 57, 59 and 63 only; magnesium and calcium at screening only. @D42 immunology bleed taken in Group 2 only. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

	C-2	CHMI	C+1-C+6	C+7	C+8	C+9	C+10	C+10.5	C+11	C+11.5	C+12	C+12.5	C+13	C+13.5	C+14	C+14.5
Attendance number - Group 1	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Timeline (days) - Group 1	-2	0	1-6	7	8	9	10	10	11	11	12	12	13	13	14	14
Window (days)	±1															
Inclusion/Exclusion criteria	X	X														
Informed consent	X	X														
Daily telephone call			X													
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urine β-hCG**																
Review contraindications	X	X														
Review AEs & medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card issued		X														
Diary card collected																
Medic alert card issued		X														
Malaria challenge		X														
Immunology (mL)	80			60											60	
Serum storage (mL)	4															
qPCR/GctPCR (mL)	2			2	2	2	2	2	2	2	2	2	2	2	2	2
Haematology (mL)[#]	2			2											2	
Biochemistry (mL) [##]	3			3											3	
Serum βhCG (mL)**	5			5											5	
SARS-COV-2 PCR swab	X															
Total Blood Volume (mL)^	96	0	0	72	2	2	2	2	2	2	2	2	2	2	72	2
Cumulative blood volume (mL)^	797	797	797	869	871	873	875	877	879	881	883	885	887	889	961	963

Table 8: Group 1 - Schedule of events for day before challenge to day 14 after challenge *(timeline/window are in relation to the day of challenge).* ***(X)*** *= If considered necessary. Visits continue until diagnosis; when diagnostic criteria are met, venepuncture and procedures are followed according to Table 11.* *******Physical observations will include blood pressure, pulse and temperature, plus weight at C-2 visit.* ********For females only.* ***#****Haematology will comprise full blood count, including reticulocyte count at C-2.* ***##*** *Biochemistry will include*

sodium, potassium, urea, creatinine, albumin and liver function tests. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.

	C+15	C+15.5	C+16	C+16.5	C+17	C+17.5	C+18	C+18.5	C+19	C+19.5	C+20	C+20.5	C+21	C+21.5
Attendance number - Group 1	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Timeline (days) - Group 1	15	15	16	16	17	17	18	18	19	19	20	20	21	21
Window (days)														
Inclusion/Exclusion criteria														
Informed consent														
Daily telephone call														
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urine β-hCG**														
Review contraindications														
Review AEs & medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card issued														
Diary card collected														
Medic alert card issued														
Immunology (mL)													60	
Serum storage (mL)														
qPCR/GctPCR (mL)	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Haematology (mL)[#]													2	
Biochemistry (mL) [##]													3	
Serum βhCG (mL)**													5	
Total Blood Volume (mL)^	2	2	2	2	2	2	2	2	2	2	2	2	72	2
Cumulative blood volume (mL)^	965	967	969	971	973	975	977	979	981	983	985	987	1059	1061

Table 9: Group 1 - Schedule of events for days 15-21 after challenge *(timeline/window are in relation to the day of challenge).* ***(X)*** *= If considered necessary. Visits continue until diagnosis; when diagnostic criteria are met, venepuncture and procedures are followed according to Table 11.* ******* *Physical observations will include blood pressure, pulse and temperature.* ******** *For females only.* ***#*** *Haematology will comprise full blood count only.* ***##*** *Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

	C+22	C+22.5	C+23	C+23.5	C+24	C+24.5	C+25	C+25.5	C+26	C+26.5	C+27	C+27.5
Attendance number – Group 1	47	48	49	50	51	52	53	54	55	56	57	58
Timeline (days) – Group 1	22	22	23	23	24	24	25	25	26	26	27	27
Window (days)												
Inclusion/Exclusion criteria												
Informed consent												
Daily telephone call												
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urine β-hCG**												
Review contraindications												
Review AEs & medications	X	X	X	X	X	X	X	X	X	X	X	X
Diary card issued												
Diary card collected												
Medic alert card issued												
Immunology (mL)												
Serum storage (mL)												
qPCR/GctPCR (mL)	2	2	2	2	2	2	2	2	2	2	2	2
Haematology (mL)[#]												
Biochemistry (mL) [##]												
Serum βhCG (mL)**												
Total Blood Volume (mL)^	2	2	2	2	2	2	2	2	2	2	2	2
Cumulative blood volume (mL)^	1063	1065	1067	1069	1071	1073	1075	1077	1079	1081	1083	1085

Table 10: Group 1 - Schedule of events for days 22-27.5 after challenge *(timeline/window are in relation to the day of challenge).* ***(X)*** *= If considered necessary. Visits continue until diagnosis; when diagnostic criteria are met, venepuncture and procedures are followed according to Table 11.* ******* *Physical observations will include blood pressure, pulse and temperature.* ******** *For females only.* ***#*** *Haematology will comprise full blood count only.* ***##*** *Biochemistry will include sodium, potassium, urea, creatinine, albumin, and liver function tests. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

	DOD/C+28 (if undiagnosed)$+	T+1 (24hr dose)^^	T+2 (48hr dose)^^	T+6	T+9	C+56	C+96@	C+276
Attendance number – Group 1	59	60	61	62	n/a	63	64	65
Timeline (days) – Group 1	-	-	-	-		56	96	276
Window (days)				±1	±2	±14	±14	±14
Telephone call					X			
Physical observations*	X	X	X	X		X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)		(X)	(X)	(X)
Urine β-hCG**	X							
Review contraindications	X							
Review AEs & medications	X	X	X	X		X	X	X
Diary card issued								
Diary card collected				X				
Medic alert card issued								
Immunology (mL)	70					80	70	70
Serum storage (mL)							4	
HIV, HBV, HCV, EBV, CMV serology (mL)+++							5	
qPCR/GctPCR (mL)	2	2	2					
Haematology (mL)#	2			2		2	2	
Biochemistry (mL) ##	3			3		3	3	
Serum βhCG (mL)**	5							
SARS-COV-2 PCR swab	X							
Total Blood Volume (mL)^	82	2	2	5	n/a	85	84	70
Cumulative blood volume (mL)^	1167	1169	1171	1176		1261	1345	1415

Table 11: Group 1 - Schedule of events from diagnosis to 276 days after challenge *(timeline/window are in relation to the day of challenge or treatment day).* ***DOD*** *= day of diagnosis;* ***C*** *= challenge;* ***T*** *= treatment;* ***(X)*** *= If considered necessary. *Physical observations will include blood pressure, pulse and temperature. **For females only. #Haematology will comprise full blood count only. ## Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests. $ if diagnosed at C+14.5 or C+21.5 do not take DOD immunology bleed (will have taken full bleed at C+14 and C+21). + immediately before drug treatment. ^^ Blood draws for qPCR will continue until the volunteer has two consecutive qPCR readings with substantial reduction in genome copies per mL. +++ EBV and CMV serology will only be repeated at the C+96 timepoint if they were negative at screening. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary. @Last visit for the subset of Group 1 participants that move into Group 3, next visit for those who go into Group 3 will be as per Table 12.*

	V4	V4-D1	V4-D3	V4-D7	V4-D14
Attendance number – Group 3	65	66	67	68	69
Timeline (days) – Group 3	Approx 150 days post 3rd vaccination	1 day post 4th vaccination	3 days post 4th vaccination	7 days post 4th vaccination	14 days post 4th vaccination
Window (days)	-7/+14	±1	-1/+2	-2/+4	-3/+5
Inclusion/Exclusion criteria	X				
Informed consent	(X)				
Physical observations*	X	X	X	X	X
Medical history/examination	(X)	(X)	(X)	(X)	(X)
Urine β-hCG**	X				
Review contraindications	X				
Review AEs & medications	X	X	X	X	X
Vaccination	X				
Diary card issued	X				
Diary card collected					
Immunology (mL)	80	20	20	50	80
RNA preservation (mL)	3	3			
Haematology (mL)#	2	2	2	2	2
Biochemistry (mL) ##	3	3	3	3	3
Total Blood Volume (mL)^	88	28	25	55	85
Group 3 - Cumulative blood volume (mL)^	1433	1461	1486	1541	1626

Table 12: Group 3 - 4th vaccination schedule of events

V4 *= 4th vaccinations with PvDBPII/Matrix M1, to occur approximately 1 month after C+96 visit following first CHMI* ***(X)*** *= If considered necessary, emphasising any acute complaints.* *******Physical observations will include blood pressure, pulse and temperature.* ********For females only.* ***#*** *Haematology will include full blood count.* ***##*** *Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

	C-2	CHMI	C+1-C+6	C+7	C+8	C+9	C+10	C+10.5$	C+11	C+11.5$	C+12	C+12.5$	C+13	C+13.5$	C+14	C+14.5$
Attendance number – Group 2	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Attendance number – Group 3	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
Timeline (days)	-2	0	1-6	7	8	9	10	10	11	11	12	12	13	13	14	14
Window (days)	±1															
Inclusion/Exclusion criteria	X	X														
Informed consent	X	X														
Daily telephone call			X													
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Review contraindications	X	X														
Review AEs & medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card issued		X														
Diary card collected																
Medic alert card issued		X														
Malaria challenge		X														
Immunology (mL)	80			60											60	
Serum storage (mL)	4															
qPCR/GctPCR (mL)	2			2	2	2	2	2	2	2	2	2	2	2	2	2
Haematology (mL)#	2			2											2	
Biochemistry (mL) ##	3			3											3	
Serum βhCG (mL)**	5			5											5	
SARS-COV-2 PCR swab	X															
Total Blood Volume (mL)^	96	0	0	72	2	2	2	2	2	2	2	2	2	2	72	2
Group 2 - Cumulative blood volume (mL)^	837	837	837	909	911	913	915	917	919	921	923	925	927	929	1001	1003
Group 3 - Cumulative blood volume (mL)^	1722	1722	1722	1794	1796	1798	1800	1802	1804	1806	1808	1810	1812	1814	1886	1888

Table 13: Group 2 (1st CHMI) and Group 3 (2nd CHMI) - Schedule of events for day before challenge to day 14 after challenge *(timeline/window are in relation to the day of challenge). Visits continue until diagnosis; when diagnostic criteria are met, venepuncture and procedures are followed according to Table 15. $From day 10 post challenge onwards, visits will continue once a day in mornings, increasing to twice daily if the malaria qPCR count is >1000 genome copies/ml.* ***(X)*** *= If considered necessary.* ******* *Physical observations will include blood pressure, pulse and temperature, plus weight at C-2 visit.* ******** *For females only.* ***#*** *Haematology will comprise full blood count only.* ***##*** *Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

	C+15	C+15.5$	C+16	C+16.5$	C+17	C+17.5$	C+18	C+18.5$	C+19	C+19.5$	C+20	C+20.5$
Attendance number - Group 2	33	34	35	36	37	38	39	40	41	42	43	44
Attendance number - Group 3	86	87	88	89	90	91	92	93	94	95	96	97
Timeline (days)	15	15	16	16	17	17	18	18	19	19	20	20
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Review AEs & medications	X	X	X	X	X	X	X	X	X	X	X	X
Diary card issued												
Diary card collected												
Medic alert card issued												
Immunology (mL)												
Serum storage (mL)												
qPCR/GctPCR (mL)	2	2	2	2	2	2	2	2	2	2	2	2
Haematology (mL)#												
Biochemistry (mL) ##												
Serum βhCG (mL)**												
Total Blood Volume (mL)^	2	2	2	2	2	2	2	2	2	2	2	2
Group 2 - Cumulative blood volume (mL)^	1005	1007	1009	1011	1013	1015	1017	1019	1021	1023	1025	1027
Group 3 - Cumulative blood volume (mL)^	1890	1892	1894	1896	1898	1900	1902	1904	1906	1908	1910	1912

Table 14: Group 2 (1st CHMI) and Group 3 (2nd CHMI) - Schedule of events from day 15 - 20 after challenge *(timeline/window are in relation to the day of challenge). Visits continue until diagnosis; when diagnostic criteria are met, venepuncture and procedures are followed according to Table 15. $From day 10 post challenge onwards, visits will continue once a day in mornings, increasing to twice daily if the malaria qPCR count is >1000 genome copies/ml.* ***(X)*** *= If considered necessary. *Physical observations will include blood pressure, pulse and temperature. **For females only. #Haematology will comprise full blood count only. ## Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

	DOD/C+21 (if undiagnosed)$+	T+1 (24hr dose)^^	T+3	T+6	T+9	C+56	C+96	C+276
Attendance number - Group 2	45	46	47	48		49	50	51
Attendance number - Group 3	98	99	100	101	n/a	102	103	104
Timeline (days)	-	-	-	-		56	96	276
Window (days)				±1	±2	±14	±14	±14
Telephone call					X			
Physical observations*	X	X	X	X		X	X	X
Medical history/ examination	(X)	(X)	(X)	(X)		(X)	(X)	(X)
Urine β-hCG**	X							
Review contraindications	X							
Review AEs & medications	X	X	X	X		X	X	X
Diary card issued								
Diary card collected				X				
Immunology (mL)	70					80	70	70
Serum storage (mL)							4	
HIV, HBV, HCV, EBV, CMV serology (mL)+++							5	
qPCR/GctPCR (mL)	2	2	2					
Haematology (mL)#	2			2		2	2	
Biochemistry (mL) ##	3			3		3	3	
Serum βhCG (mL)**	5							
SARS-COV-2 PCR swab	X							
Total Blood Volume (mL)^	82	2	2	5	n/a	85	84	70
Group 2 - Cumulative blood volume (mL)^	1109	1111	1113	1118	n/a	1203	1287	1357
Group 3 - Cumulative blood volume (mL)^	1994	1996	1998	2003	n/a	2088	2172	2242

Table 15: Group 2 (1st CHMI) and Group 3 (2nd CHMI) - Schedule of events from diagnosis to 276 days after challenge *(timeline/window are in relation to the day of challenge or treatment day).* ***DOD*** *= day of diagnosis;* ***C*** *= challenge;* ***T*** *= treatment;* ***(X)*** *= If considered necessary. *Physical observations will include blood pressure, pulse and temperature. **For females only. #Haematology will comprise full blood count only. ## Biochemistry will include sodium, potassium, urea, creatinine, albumin and liver function tests. $ if diagnosed at C+14.5 do not take DOD immunology bleed (will have taken full bleed at C+14). + immediately before drug treatment. ^^ Blood draws for qPCR will continue until the volunteer has two consecutive qPCR readings with substantial reduction in genome copies per mL. +++ EBV and CMV serology will only be repeated at the C+96 timepoint if they were negative at screening. ^Blood volumes as given in the table are maximal volumes and smaller blood volumes may be taken at any visit, at the discretion of the investigator, with the exception of additional blood volume that may be required for any additional tests that may be performed if deemed clinically necessary.*

12 ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study. The safety profile will be assessed on an on-going basis by the Investigators. The Chief investigator, Principal Investigator, and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

12.1 Definitions

12.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a volunteer, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Each participant reported adverse event will be graded by the participant according to the table for grading severity of adverse events (see section 12.3. Severity gradings may be reviewed and discussed with the participants at the clinic visits.

12.1.2 Adverse Reaction (AR)

An AR is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by the reporting medical Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

12.1.3 Unexpected Adverse Reaction

An unexpected adverse reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

12.1.4 Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death.
- Life-threatening event (i.e. the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation or prolongation of hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive

treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.

- Congenital anomaly or birth defect.

12.1.5 Serious Adverse Reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting Investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, including *P. vivax* CHMI, based on the information provided.

12.1.6 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the IB, non-investigational medicinal product dossier (for *P. vivax* blood inoculum) or Summary of Product Characteristics (SmPC).

12.1.7 Foreseeable Adverse Reactions

The foreseeable ARs following vaccination with PvDBPII-Matrix M1 include injection site pain, erythema, warmth, swelling, pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea. These adverse events will be listed as solicited adverse events providing they occur within 7 days of the day of vaccination. AEs other than these, or those AEs occurring outside of the 7 days after vaccination, will be listed as unsolicited adverse events.

12.1.8 Other Foreseeable Medical Occurrences

The following medical occurrences are foreseeable:

- Clinical *P. vivax* disease resulting in one or more of fever, tachycardia, hypotension, feverishness, chills, rigor, sweats, headache, anorexia, nausea, vomiting, myalgia, arthralgia, low back pain, fatigue, lymphopenia and thrombocytopenia;
- Clinical *P. vivax* disease resulting in inpatient transfer of care to the NHS;
- Adverse reactions to Riamet®, Malarone, paracetamol or cyclizine, as detailed in the SmPCs for these medications.

12.1.9 Expected Serious Adverse Events

No serious adverse events are expected in this study.

12.2 Causality Assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI or the CI-delegated clinician at the coordinating site (Oxford). An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy (Table 16). Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, for which causality should be assigned by the reporting Investigator.

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

Table 16: Guidelines for assessing the relationship of vaccine administration to an AE.

12.3 Reporting Procedures for All Adverse Events

All AEs occurring in the 28 days following each vaccination observed by the Investigator or reported by the volunteer, whether or not attributed to study medication, will be recorded. Recording and reporting of all AEs will take place as detailed in SOP VC027. All AEs occurring in post-CHMI, until 6 days after completion of antimalarial treatment (i.e. 9 days after treatment initiation) will also be recorded- these will be entered directly into the eCRF. We will NOT record bruising due to blood-taking or cannulation. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, the event is considered stable or until a non-study related causality is assigned (if the volunteer consents to this). Serious adverse events (SAEs) will be collected throughout the entire trial period.

Each adverse event will be graded by the participant according to the table for grading severity of adverse events (see Table 17). Severity gradings may be reviewed and discussed with the participants at the clinic visits. Volunteers will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

The severity of clinical adverse events will be assessed by the Investigators according to the scales in Tables 17-19.

Severity grading for laboratory AEs are dependent on the OUH laboratory's reference and will be graded according to the scales in the site-specific table that can be found in the Investigator file. These ranges will be based on FDA guidance relative to local laboratory reference ranges [112].

CRP may be measured as part of the biochemistry profile on days 0, 1, 3, 7, 56, 57, 59 and 63. However, CRP results will be used in relation to exploratory immunological analysis only, and therefore, any abnormal result will not be considered an AE and grading scales for CRP are not listed in the site-specific table, i.e. CRP results will not be considered within the safety analysis.

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 17: Severity grading criteria for AEs.

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	>38.0	>39.0

Table 18: Severity grading criteria for clinically significant abnormal physical observations. 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).

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
Erythema at injection site*	1	>3 - ≤50 mm
	2	>50 - ≤100 mm
	3	>100 mm
Swelling at injection site*	1	>3 - ≤20 mm
	2	>20 - ≤50 mm
	3	>50 mm

erythema or swelling ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event.

Table 19: Severity grading criteria for local adverse events.

12.4 Reporting Procedures for Serious AEs (see SOP OVC005 Safety Reporting)

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported on the SAE forms to members of the study team immediately after the Investigators become aware of their occurrence, as described in SOP OVC005. Copies of all reports will be forwarded for review to the Chief Investigator and Principal Investigators (as the Sponsor's representatives) within 24 hours of the Investigator being aware of the suspected SAE. The local safety monitor (LSM) will be notified of SAEs which are deemed possibly, probably or definitely related to study interventions; the LSM will be notified immediately (within 24 hours) of the Investigators' being aware of their occurrence. SAEs will not normally be reported immediately to the REC 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 volunteers, at the discretion of the Chief Investigator and/or LSM. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

12.4.1 Reporting Procedures for SUSARS

The Chief Investigator will report all SUSARs to the MHRA and REC within required timelines (15 days for all SUSARs, unless life threatening or fatal in which case 7 days, with a final report within a further 8 days (total 15)). The Chief Investigator will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

All SUSARs and deaths occurring during the study will be reported to the Sponsor. For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

12.5 Development Safety Update Report

A Development Safety Update Report (DSUR) will be submitted by the Sponsor, or delegate, to the competent authority and ethics committee on the anniversary of the first approval date from the regulatory authority for each IMP.

12.6 Adverse Events of Special Interest

Adverse events of special interest will be reported as SAEs. These are:

- Severe hypersensitivity reactions (e.g. anaphylaxis)
- Any new, suspected auto-immune disease

12.7 Procedures to be followed in the event of abnormal findings

Eligibility for enrolment in the trial in terms of laboratory findings will be assessed as detailed in SOP VC027. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. Laboratory adverse events will be assessed using the site-specific tables in the Investigator site file (ISF)/ trial master file (TMF). If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate and with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

12.8 Local Safety Monitor

An independent Local Safety Monitor (LSM) will be appointed to provide real-time safety oversight. The LSM will review SAEs deemed possibly, probably or definitely related to study interventions. The LSM will be notified within 24 hours of the Investigators' being aware of their occurrence. The LSM has the power to terminate the study if deemed necessary following a study intervention-related SAE. At the time of writing, the LSM will be Prof Brian Angus, a Clinical Tutor in Medicine, Honorary Consultant Physician and Director, Centre for Tropical Medicine at the University of Oxford. All correspondence between the Investigator and the LSM will be conveyed by the Investigator to the trial Sponsor on their request

The LSM may be contacted for advice and independent review by the Investigator or trial Sponsor in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to a study intervention
- Any other situation where the Investigator or trial sponsor feels independent advice or review is important.

The study can be put on hold upon advice of the Local Safety Monitor, Chief Investigator, Study Sponsor or Ethical Committee(s) for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the subjects or the reliability of the data. If the study is placed on hold it may only be restarted following discussion with and approval from the LSM, the ethics committee(s), the trial Sponsor and Chief Investigator.

12.9 Safety stopping/holding rules

Safety holding rules have been developed considering the fact that this is a first-in-human study. 'Solicited adverse events' are those listed as foreseeable adverse reactions in the protocol, occurring within the first 7 days after vaccination (day of vaccination and six subsequent days).

'Unsolicited adverse events' are adverse events other than the foreseeable ARs occurring within the first 7 days, or any AEs occurring after the first 7 days after vaccination.

12.9.1 Group holding rules

If a group holding rule is activated, then further vaccinations will not occur until an internal safety review has been conducted and it is deemed appropriate to restart dosing. The regulatory authority must be informed and a request to restart dosing with pertinent data must be submitted as a request for a substantial amendment. The internal safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Participant Information Sheet (PIS) are discussed.
- New, relevant safety information from ongoing research programmes on the various components of the vaccine (i.e. the Matrix-M1 adjuvant).

The local ethics committee and Adjuvant System manufacturers (Novovax) will also be notified if a holding rule is activated or released.

All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

The holding rules are as follows:

- **Solicited local adverse events:**
    - If more than 25% of doses of a vaccine are followed by the same Grade 3 solicited local adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 72 hrs.
- **Solicited systemic adverse events:**
    - If more than 25% of doses of a vaccine are followed by the same Grade 3 solicited systemic adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 48 hrs.
- **Unsolicited adverse events:**
    - If more than 25% of volunteers develop the same Grade 3 unsolicited adverse event (including the same laboratory adverse event) that is considered possibly, probably or definitely related to vaccination and persists at Grade 3 for > 48 hrs.
- **A serious adverse event considered possibly, probably or definitely related to vaccination occurs**

12.9.2 Individual stopping rules

In addition to the above stated group holding rules, stopping rules for individual volunteers will apply (i.e., indications to withdraw individuals from further vaccinations).

- **Local reactions:** Injection site ulceration, abscess or necrosis.
- **Laboratory AEs:**

- The volunteer develops a Grade 3 laboratory adverse event considered possibly, probably or definitely related within 7 days after vaccination and persisting continuously at Grade 3 for >72hrs.

- **Solicited systemic adverse events:**
    - The volunteer develops a Grade 3 systemic solicited adverse event considered possibly, probably or definitely related within 2 days after vaccination (day of vaccination and one subsequent day) and persisting continuously at Grade 3 for >72hrs.

- **Unsolicited adverse events:**
    - The volunteer has a Grade 3 adverse event, considered possibly, probably or definitely related to vaccination, persisting continuously at Grade 3 for >72hrs.
    - The volunteer has a serious adverse event considered possibly, probably or definitely related to vaccination.
    - The volunteer has an acute allergic reaction or anaphylactic shock following the administration of the vaccine investigational product.

If a volunteer has an acute illness (moderate or severe illness with or without fever) or a fever (oral temperature greater than 37.5°C) at the scheduled time of administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated volunteers will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

In addition to these pre-defined criteria, the study can be put on hold upon advice of the Local Safety Monitor (LSM), Chief Investigator, Study Sponsor, regulatory authority, Research Ethics Committee (REC) or Local Safety Monitor (LSM), for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the volunteers or the reliability of the data.

13 STATISTICS

13.1 Sample size

This is an open label, single site, first-in-human, Phase I/IIa, blood-stage *P. vivax* malaria vaccine trial aiming to assess the safety, immunogenicity and efficacy of the PvDBPII-Matrix M1 vaccine candidate against blood-stage challenge for the first time, utilising a newly produced cryopreserved inoculum source. The number of volunteers undergoing vaccination followed by malaria challenge will be 10-12.

Outcome measures will be assessed as described in Section 7. PMR will constitute the primary endpoint for the study and pooled data for PMR will be compared with PMR in 10-12 malaria-naïve infectivity controls, recruited as part of another study, VAC069, who will undergo CHMI in parallel to the volunteers in VAC079. Any statistically significant change in PMR in a vaccine group would be considered as success, however, it is not clear what level of change in PMR would be likely to be significant in terms of clinical outcome. As this is only the third CHMI study with this new bank of *Plasmodium vivax*, there is insufficient data to allow accurate modelling/power calculations, but it is considered that ten volunteers in the vaccinated group will be sufficient to allow detection of statistically significant differences in PMR between vaccinees and controls

14 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

14.1 Investigator procedures

Approved site-specific SOPs will be used at all clinical and laboratory sites.

14.2 Monitoring

Monitoring will be performed by CTRG using established procedures, according to ICH Good Clinical Practice (GCP). Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol and GCP. The sites will provide direct access to all trial related source data/documents and reports for the purpose of auditing by the Sponsor and inspection by local and regulatory authorities.

14.3 Modification to protocol

No substantial amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any substantial amendments to the trial that appear necessary during the course of the trial must be discussed by the Investigator and Sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Chief Investigator and will be made a formal part of the protocol following ethical approval.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which ethical committee(s) approval has already been given, are not initiated without review and approval from the ethics committee(s) and regulatory authorities except to eliminate apparent immediate hazards to the subject.

14.4 Protocol deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the site trial master file.

14.5 Audit & inspection

The QA manager performs system based internal audits to check that trials are being conducted, data recorded, analysed and accurately reported according to study protocols, departmental SOPs and in compliance with ICH GCP. The audit schedule includes laboratory activities. The internal audits will supplement the external monitoring process and will review processes not covered by the external monitor.

The Sponsor, trial sites, ethical committee(s), and authorised individuals may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the regulatory authority to ensure compliance with protocol and national regulations. The Sponsor will assist in any inspections.

14.6 Serious breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to effect to a significant degree –

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

(b) the scientific value of the trial.

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 REC committee, Regulatory authority and the relevant NHS host organisation within seven calendar days.

14.7 Trial progress

The progress of the trial will be overseen by the Chief Investigator.

14.8 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 ICMJE guidelines and other contributors will be acknowledged. Data may also be used for a PhD or MD thesis.

14.9 Intellectual Property

Ownership of intellectual property (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

15 ETHICS

15.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki 2008.

15.2 ICH guidelines for good clinical practice

The Investigator will ensure that this study is conducted in full conformity to Medicine for Human use (Clinical Trials) Regulations 2004 (SI 2004 No. 1031) and its amendments and with the ICH guidelines for GCP (CPMP/ICH/135/95) July 1996.

15.3 Approvals

Following Sponsor approval, a copy of the protocol, proposed informed consent form, other written volunteer information and the proposed advertising material will be submitted to an appropriate REC and the HRA for written approval. The Investigator will submit and, where necessary, obtain approval from the REC and the HRA for all subsequent amendments to the protocol and associated trial documents. A non-substantial amendment does not require REC approval, however, the REC will be notified in the event of any such change. The Investigator will notify deviations from the protocol or SAEs occurring at the site to the Sponsor and will notify the REC(s) of these if necessary in accordance with procedures.

15.4 Reporting

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

15.5 Volunteer confidentiality

All data will be de-identified; volunteer data will be identified only by a unique study number in the CRF and database. Separate confidential files containing identifiable information will be stored in secured locations. Only the Sponsor representative, Investigators, the clinical monitor, the ethical committee(s) and the regulatory authorities will have access to the records. Photographs taken (if required, with the volunteer's written, informed consent) will not include the volunteer's face and will be identified by the volunteer's trial specific identification number only. Once developed, photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

Results of any COVID-19 swab tests carried out through this study will be legally required to be reported to Public Health England. Along with the result of the COVID-19 test, the following details will also reported: personal details of the participant (name, date of birth, contact details, ethnicity, NHS number) and date of diagnosis and symptom onset if applicable.

16 DATA HANDLING AND RECORD KEEPING

16.1 Data handling

The Chief Investigator will be the data manager with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. Data will be entered into the volunteers' CRFs in a paper and/or electronic format (using the OpenClinica™ database). Electronic data will be stored on secure servers which are outsourced by OpenClinica™. OpenClinica™ meets FDA part 11B standards. This includes safety data, laboratory data and outcome data. Data are entered in a web browser on PCs and then transferred to the OpenClinica Database by encrypted (https) transfer. Safety data will also be collected through an electronic diary, which is stored on a secure server.

16.2 Record keeping

The Investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Chief Investigator, co-investigators, clinical research nurses and authorised personnel will have access to records. The investigators will permit authorised representatives of the Sponsor, ethical committee(s), regulatory agencies, and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

16.3 Source data and electronic case report forms (eCRFs)

All protocol-required information will be collected in the electronic diaries and eCRFs designed by the Investigator. All source documents will be filed in the participants' notes. Source documents are original documents, data, and records from which the volunteer's eCRF data are obtained. For this study these will include, but are not limited to; volunteer consent form, blood results, GP response letters, laboratory records and correspondence. In the majority of cases, electronic diaries and eCRF entries will be considered source data as these are the site of the original recording (i.e. there is no other written or electronic record of data). In this study this will include, but is not limited to medical history, medication records, vital signs, physical examination records, urine assessments, blood results, adverse event data and details of study interventions. All source data and volunteer CRFs will be stored securely.

16.4 Data protection

The trial will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the Sponsor.

Results of any COVID-19 swab tests carried out through this study will be legally required to be reported to Public Health England. Along with the result of the COVID-19 test, the following details will also reported: personal details of the participant (name, date of birth, contact details, ethnicity, NHS number) and date of diagnosis and symptom onset if applicable.

17 FINANCING AND INSURANCE

17.1 Financing

The study is funded through a grant from The Wellcome Trust.

17.2 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). NHS indemnity operates in respect of the clinical treatment that is provided.

17.3 Compensation

Volunteers will be compensated for their time and for the inconvenience caused by procedures as below.

- Screening visit £25

Subsequent visits:

- Travel expenses £15 per visit
- Inconvenience of blood tests: £10 per blood donation
- Time required for visit: £20 per hour
- Admission (Illness) Compensation £480 total

Where travel expenses are greater than £15 per visit because the volunteer lives outside the city of the trial site, the volunteer may be given further reimbursement to meet the cost of travel necessary for study visits at the Investigator's discretion.

	Time in Trial (approx.)	Maximum No. of Visits	Maximum Volume of Blood Taken (mL)	Compensation (up to approximately)
Group 1	2 years	65	1415	£3500
Group 2	1 year	51	1357	£2890
Group 3	9 months	39	897	£2355
total including Group 1 visits	2.5 years	104	2242	£5810

Table 20: Estimated compensation amounts**.**

18 APPENDICES

Appendix A: laboratory values for exclusion

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 excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory adverse event, according to the laboratory adverse event tables (filed in the TMF), on repeat of an abnormal test result. Urinalysis at screening will be assessed as per the table below:

URINE ANALYSIS (using MULTISTIX)	
Protein*	2+ or Protein creatinine ratio of ≥ 50 mg/mmol
Blood[£]	2+ on two dipstick tests
Glucose	1+

Table 21: Urinalysis assessment.

*In the event of the dipstick testing positive for protein with ≥1+ protein urine should be sent for a protein creatinine ratio.

[£] In the event of urine dipstick testing positive for ≥1+ blood with, or without, protein, a repeat dipstick test will be carried out to confirm haematuria. In female volunteers, a menstrual history will be taken to elicit whether the subject is currently menstruating and if they are, urine dipstick will be repeated after 1 - 2 weeks. If blood and/or proteinuria persist in any volunteer, an interpretation of the results will be undertaken by the Investigator on an individual basis to determine if they will be excluded from the trial, and the appropriate follow-up arranged.

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