PART A: PROJECT INFORMATION

A1. TITLE OF THE STUDY IN THAI AND ENGLISH

English: A Phase II Clinical Study to Assess the Safety, Immunogenicity, and Efficacy of Blood-stage *Plasmodium Vivax* Malaria Vaccine Candidate PvDBPII/Matrix-M™ in Healthy Thai Adults Living in Thailand, controlled human malaria infection (CHMI)

Study Code: MAL22004

Trial Registry Name: ClinicalTrials.gov; ClinicalTrials.gov ID: NCT05380388

A2. RESEARCH PROPOSAL VERSION DATE: V.3.0, dated 14 December 2022

Type of submission ☐ Initial review (first time submission)

☑ Revision according to TMEC suggestions No…2..

☐ Amendment No. ….…

A3. PRINCIPAL INVESTIGATOR NAME: Professor Nicholas Day and Research Professor Dr. Jetsumon Sattabongkot Prachumsri

☑ Faculty Staff (Go to A4.1)

☐ **Student, ID**……..… (Please check the following, and go to A4.2)

☐ Research for Thesis ☐ Research for Thematic Paper

☐ M.Sc. (Trop. Med.) ☐ M.C.T.M.

☐ Ph.D. (Trop. Med.) ☐ M.C.T.M. (T.P.)

☐ Ph.D. (Clin. Trop. Med.) ☐ Other, specify ……

☑ **Other** Mahidol Oxford Tropical Medicine Research Unit (MORU) Staff

(Please specify, and go to A4.1)

A4. LIST NAME, AFFILIATION AND CONTACT DETAILS OF ALL INVESTIGATORS

A4.1 For Faculty staff and other

Name	Position	Contact address	E-mail address
Professor Nicholas Day	Co-Principal Investigator	Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	nickd@tropmedres.ac
Research Professor Dr. Jetsumon Sattabongkot Prachumsri	Co-Principal Investigator	Mahidol Vivax Research Unit (MVRU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	jetsumon.pra@mahidol.ac.th
Assoc. Professor Kittiyod Poovorawan	Accountable Investigator (FTM Thai Staff) and Site-Principal Investigator	Department of Clinical Tropical Medicine Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	kittiyod.poo@mahidol.ac.th
Professor Sasithon Pukrittayakamee	Co-investigator	Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	yon@tropmedres.ac
Assist. Professor Podjanee Jittamala	Co-investigator	Department of Tropical Hygiene Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	podjanee@tropmedres.ac

Name	Position	Contact address	E-mail address
Assist. Professor Borimas Hanboonkunupakarn	Co-investigator	Department of Clinical Tropical Medicine Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	borimas@tropmedres.ac
Professor Sir Nicholas White	Co-investigator	Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	nickwdt@tropmedres.ac
Professor Arjen Dondorp	Co-investigator	Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	arjen@tropmedres.ac
Dr. Angela M Minassian	Co-investigator	Centre for Clinical Vaccinology and Tropical Medicine, Department of Biochemistry, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE	angela.minassian@bioch.ox.ac.uk
Professor Philip Bejon	Co-investigator	Kemri-Wellcome Trust research program Kilifi, Kenya	pbejon@kemri-wellcome.org
Professor Simon J Draper	Co-investigator	Department of Biochemistry, University of Oxford, Dorothy Crowfoot Hodgkin Building, South Parks Road, Oxford, OX1 3QU, United Kingdom	Simon.draper@bioch.ox.ac.uk
Professor Mallika Imwong	Co-investigator	Molecular Tropical Medicine and Genetics Faculty of Tropical Medicine	mallika.imw@mahidol.ac.th

Name	Position	Contact address	E-mail address
		Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	
Assist. Prof. Wang Nguitragool	Co-investigator	Molecular Tropical Medicine and Genetics Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	Wang.ngu@mahidol.edu
Professor Kesinee Chotivanich	Co-investigator	Mahidol Oxford Tropical Medicine Research Unit (MORU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	nok@tropmedres.ac
Dr. Wanlapa Roobsoong	Co-investigator	Mahidol Vivax Research Unit (MVRU) Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	wanlapa.ros@mahidol.edu
Assist. Prof. Patchara Sriwichai	Co-investigator	The Department of Medical Entomology Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	patchara.sri@mahidol.ac.th
Assoc. Professor Pornsawan Leaungwutiwong	Co-investigator	Tropical Medicine Diagnostic Reference laboratory Department of Microbiology and Immunology Faculty of Tropical Medicine Mahidol University 420/6 Rajvithi Road Bangkok 10400 Thailand	pornsawan.lea@mahidol.ac.th

A4.2 For students: Not applicable

Name	Position	Contact address	E-mail address
	Principal Investigator		

	Accountable Investigator (Student/Advisor)		
	Advisor		
	Co-advisor		
	Co-advisor		

A5. RESPONSIBILITY OF PI

☑ Whole study

☐ Partial responsibility

A6. NUMBER OF YOUR ONGOING RESEARCH PROJECT(S)

1) Professor Nicholas Day: 5 studies
2) Research Professor Dr. Jetsumon Sattabongkot Prachumsri: 6 studies

A7. CONTACT PERSON

1. Professor Nicholas Day (Co-Principal Investigator)
   Mahidol Oxford Tropical Medicine Research Unit (MORU)
   Faculty of Tropical Medicine
   Mahidol University
   420/6 Rajvithi Road
   Bangkok 10400 Thailand
   Email: nickd@tropmedres.ac

2. Research Professor Dr. Jetsumon Sattabongkot Prachumsri (Co-Principal Investigator)
   Mahidol Vivax Research Unit (MVRU)
   Faculty of Tropical Medicine
   Mahidol University
   420/6 Rajvithi Road
   Bangkok 10400 Thailand
   Tel. 02 306 9187
   Email: jetsumon.pra@mahidol.ac.th
   Mobile phone: 08 1611 4241

3. Assoc. Professor Kittiyod Poovorawan (Accountable Investigator and Site-Principal Investigator)
   Department of Clinical Tropical Medicine
   Faculty of Tropical Medicine

Mahidol University
420/6 Rajvithi Road
Bangkok 10400 Thailand
Email: kittiyod.poo@mahidol.ac.th
Mobile phone: (083) 149-6864

A8. NATURE OF THE STUDY- STUDY INVOLVING SPECIMEN COLLECTION

☑ Clinical trial phase II -…/ Intervention

☐ Bioequivalence/ pharmacokinetic drug study

☐ Prospective epidemiological research

☐ Laboratory study

A9. IS THE PROJECTS A SINGLE CENTER OR MULTI-CENTER?

☑ Single center

☐ Multicenter (within Thailand)

Please specify the study sites …….....

☐ Multicenter (International)

Please specify the study sites …….....

A10. PROJECT SUMMARY IN THAI AND ENGLISH

A10.1 Full protocol summary in English (not more than 1,000 words per language)

This project is the third part of a 5-year research programme entitled "Malaria Infection Studies in Thailand (MIST)", and is known as MIST3. MIST3's primary objectives are to assess the safety and efficacy of the PvDBPII/Matrix-M^{TM} vaccine candidate in healthy adult Thai participants and to establish whether the PvRII/Matrix-M^{TM} vaccine can demonstrate a reduced parasite multiplication rate in vaccinated participants compared to a control group (placebo vaccine) in a blood-stage controlled human malaria infection model. This study will recruit up to 46 eligible healthy participants aged 20-55 in Thailand at the Faculty of Tropical Medicine, Mahidol University. Twenty-three participants will receive three doses of the PvDBPII/Matrix-M^{TM} candidate vaccine, and 23 participants will receive three doses of the placebo vaccine (normal saline). Safety and immunogenicity will be evaluated after each dose as per protocol.

Approximately 3-4 weeks after receiving the third vaccination, all participants will undergo blood-stage CHMI with *Plasmodium vivax*. The participants will be monitored closely as in-patients during inoculation and after the parasite was detected or clinically indicated in the Hospital for Tropical Diseases and treated according to the Research Proposal Submission Form.

A10.2 Part of study conducted by PI

☑ Whole Study

☐ Part of the Study

A11. SOURCE(S) OF FUNDING/ SPONSOR(S) AND BUDGET (INFORMATION REQUIRED FOR REVIEW AND CONSIDERATION)

☑ Funded by: Wellcome Trust

Budget amount: 6,000,000 THB

☐ Expecting fund from: ……

Budget amount: ……..…….…….

A12. DECLARE CONFLICT OF INTEREST

The Co-Principal Investigators and the Investigators declare they have no conflict of interest.

PART B: DETAILS OF THE STUDY (Describe only the responsibilities of the PI)

B1. BACKGROUND AND RATIONALE

The *P. vivax* malaria problem

Plasmodium vivax (P. vivax) is one of five *Plasmodium* species that cause human malaria and accounts for the most cases of non- falciparum malaria worldwide. It is now the most widespread human malarial infection in endemic areas outside Africa. The main reasons for the wide geographical distribution of *P. vivax* are likely to be related to the following aspects of *P. vivax* biology: (1) its ability to **relapse** from the dormant liver stage (hypnozoite), (2) its **high transmission potential** due to early production of gametocytes, high infectivity to mosquitoes and shorter development cycle in the vector - host compared to other plasmodia (1, 2).

P. vivax has been considered as causing 'benign' malaria, but recent large case series have demonstrated that *P. vivax* infection is associated with significant morbidity and mortality (3). Clinical cases are not only due to primary infection following the infected mosquito bite but also

relapses from the hypnozoite stage, which occur weeks to years after primary infection (4). Standard schizonticidal regimens are not effective against the hypnozoite. Radical cure of the hypnozoite stage requires therapy with one of the 8-aminoquinolines, primaquine and tafenoquine. However, both medications carry a significant risk of severe haemolytic anaemia in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) (5). There is no cheap, reliable point-of-care G6PD test, so many *P. vivax* patients do not currently receive adequate anti-hypnozoite treatment. More recently, the importance of the cytochrome P450 enzyme CYP2D6 in the metabolism of primaquine to the active metabolite has been recognized (6, 7). A common polymorphism in the CYP2D6 gene for the cytochrome P450 enzyme results in poor conversion of primaquine to the active form, resulting in treatment failures. It is estimated that these two factors combined may make nearly 40% of the population at risk of *P. vivax* infection either ineligible for or unresponsive to primaquine therapy (5).

Recent calls for control and 'eradication' of malaria worldwide 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. Consequently, the revised Malaria Vaccine Technology Roadmap to 2030 now recognizes the importance of *P. vivax* and calls for development of 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 (8, 9). More recently, research into vivax malaria has increased with candidate vaccines being developed and taken forward to clinical trial (10-12)

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

P. vivax malaria vaccine approach

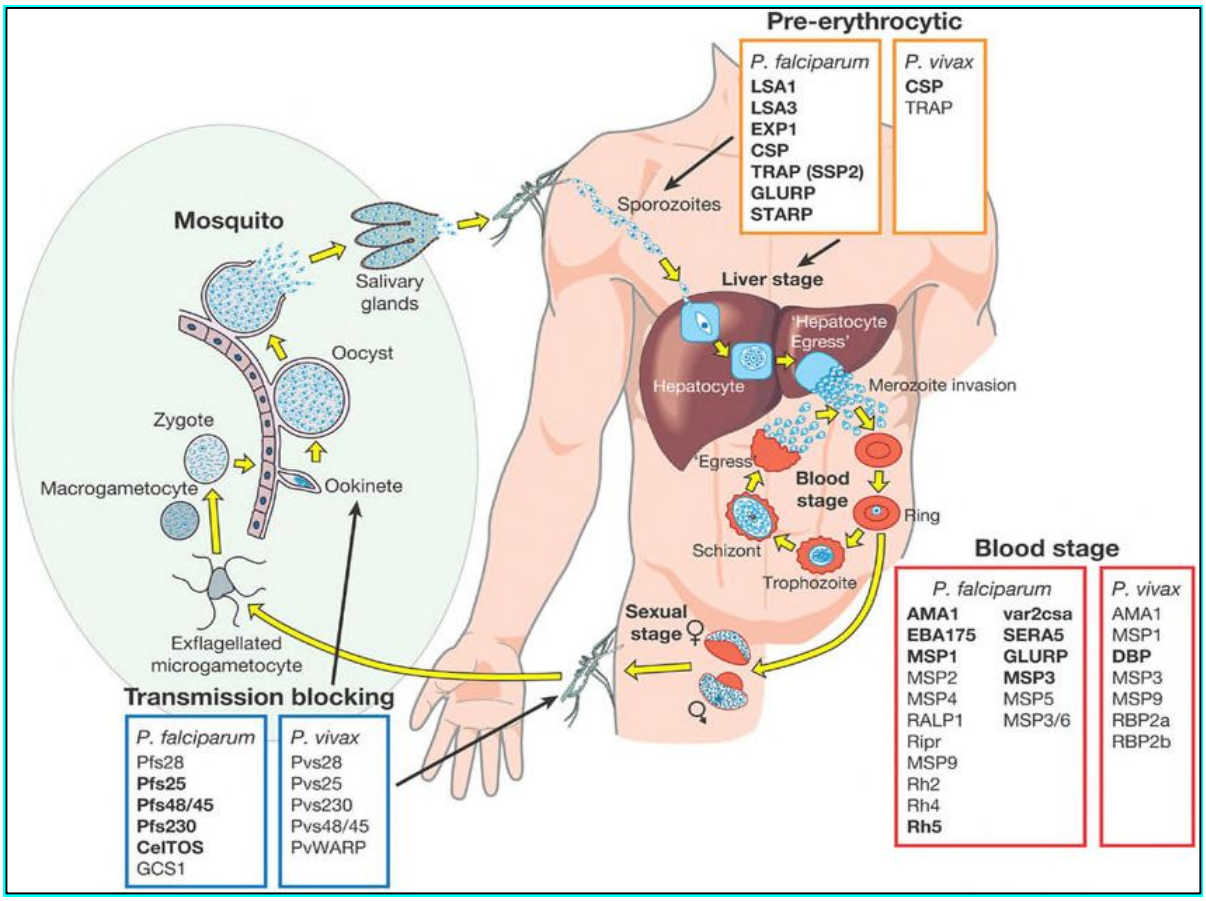
Most of the *P. vivax* malaria vaccine candidates that are currently under development target individual stages of the *P. vivax* parasite's life cycle. Several highly abundant parasite proteins have been identified as targets of natural immunity and in the recent years the list of possible candidates has expanded.

Subunit vaccines for *P. vivax* have been developed based on these candidate antigens. There are three major approaches for the development of malaria vaccines that correspond to the three stages of the parasite's life cycle as follow:

- **Pre-erythocytic antigens;** vaccine using these antigens aim to **prevent initial infection** by targeting the infective stage (sporozoites) that were introduced by the mosquito bite. Alternatively, these vaccines can target antigens expressed by liver stage parasites to prevent the emergence of merozoites into the blood stream e.g. *P. vivax* circumsporozoite protein (PVCSP), and *P. vivax* thrombospondin related adhesive protein (PvTRAP).

- **Blood stage antigens**; since all of the symptoms of malaria occur during this stage, the majority of vaccines targeting antigens expressed during the blood stage are designed primarily to **prevent disease or morbidity and mortality associated with the disease** when parasites are in red blood cell (RBC). One approach is to target **merozoite antigens** to prevent red blood cell invasion and reduce the density and prevalence of parasites in the infected host RBC e.g. receptor-binding region II of *P. vivax* Duffy binding protein (PvDBPII), *P. vivax* merozoite surface proteins (MSP 1), *P. vivax* apical merozoite antigen 1 (PvAMA1), and *P. vivax* reticulocyte binding protein (PvRBP).
- **Transmission blocking antigens;** the transmission blocking vaccines target antigens expressed during parasite lifecycle stages in the mosquito (e.g. gametocyte or oocyst antigens). Transmission blocking vaccines aim to block malaria transmission from mosquitoes to humans by **preventing the malaria parasite from developing in the mosquito**. Although these vaccines would not directly prevent infection or disease, they would assist elimination efforts through **preventing the onward transmission** of infections. Candidates include parasite proteins expressed on surface of zygote (Pvs25) and ookinete (Pvs28), and alanyl aminopeptidase 1 (AnAPN1), a highly conserved midgut surface antigen of *Anopheles* mosquitoes that is essential for ookinete invasion and development). (Table 1, Figure 1)

Figure 1. Malaria vaccine leading candidate antigens



Ref. Barry and Arnott et al. Frontiers in Immunology, 2014 (359)

Table 1. *P. vivax* malaria vaccine candidates under development [10]

Vaccine candidate	Development	Lifecycle	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-	Pre-clinical	Liver-stage	PvTRAP	Prime-boost, viral vectors
PvDBPII-DEKnull	Pre-clinical	Blood-stage	PvDBP	Rec. protein
PvDBPII	Phase I	Blood-stage	PvDBP	Rec. protein-GLA-SE
PvDBPII	Phase I/IIa	Blood-stage	PvDBP	Rec. protein- Matrix-M1™
ChAd63-PvDBPII/MVA-PvDBPII	Phase I/IIa	Blood-stage	PvDBP	Prime boost, viral vectors
PvMSP119	Pre-clinical	Blood-stage	PvMSP1	Rec. protein-Montanide ISA720
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

Blood stage *P. vivax* controlled human malaria infection experience

There have been 8 studies of blood-stage *P. vivax* human challenge. Five studies were conducted at the Queensland Institute of Medical Research (QIMR), Brisbane, Australia, and 3 studies have been recently conducted by the University of Oxford, UK (13).

In 2018, Oxford University undertook the first proof-of-concept studies in Europe of *P. vivax* human challenge. First, two healthy UK adult volunteers were safely infected with a clonal strain of *P. vivax* by the bite of infected mosquitoes transported from Thailand (VAC068, ClinicalTrials.gov: NCT03377296) (13). 250 mL blood was obtained from both volunteers at a prespecified parasitaemia/clinical threshold. Following stringent safety screening, the parasite stabilate from one of these donors (PvW1) was thawed and used to inoculate 6 healthy malaria-naive United Kingdom adults by blood-stage CHMI, at 3 different dilutions (whole vial or a neat dose, 1:5 dilution and 1:20 dilution) (VAC069A, NCT03797989). Parasitaemia developed in all volunteers, who were diagnosed within 12.5-16.5 days of infection and had detectable gametocytaemia by qPCR within 1-3 days of peak parasitaemia. They were successfully drug treated with artemether/lumefantrine or atavoquone/proguanil.

The majority of volunteers (4/6) experienced either no or mild AEs, whilst 2/6 volunteers reported malaria related clinical signs and symptoms. The majority of the AE symptoms resolved within a few days after treatment initiation. Of the laboratory AEs recorded, transient lymphocytopenia and thrombocytopenia were recorded in 4/6 and 2/6 volunteers, respectively. A transient grade 1-2 transaminitis was also seen in 4/6 volunteers at 6 days post treatment. ALT levels fully resolved to pre-challenge levels with no associated abnormalities in other indices of liver function and no associated clinical symptoms..

Based on these infectivity data, and accounting for possible variation in the number of pRBC between thawed cryopreserved vials, an inoculum dilution of 1:10 was selected for future *P. vivax* blood-stage human challenge studies. To-date, the PvW1 inoculum has been administered to a total of 19 malaria-naïve UK volunteers as a primary challenge, with 12 and 2 volunteers going on to receive a secondary and tertiary homologous repeat blood-stage challenge, respectively (VAC069 B-D re-infection studies) (12). In Thailand, the blood-stage challenge to identify optimal dilution is on going and the result should be available in the first quarter of 2023.

PvDBPII/Matrix-M™ vaccine development

PvDBPII antigen in clinical studies

The clinical manifestations of malaria are caused by the blood stage of the malaria parasite's life cycle, which involves repeated cycles of merozoite invasion, intracellular replication, and egress. The invasion of human red blood cells by *P. vivax* is mediated by interaction of the *P. vivax* Duffy-

binding protein (PvDBP) with its receptor, the Duffy antigen receptor for chemokines (DARCs) (14-17). Duffy-negative individuals are largely resistant to *P. vivax* infection (14). The functional receptor-binding domain of PvDBP, known as region II (PvDBPII), binds DARC to mediate invasion by *P. vivax* merozoites (17). Although PvDBPII is polymorphic, the DARC binding residues appear to be highly conserved across *P. vivax* strains (10). This finding implies that PvDBPII may contain cross-reactive epitopes that trigger strain-transcending inhibitory antibodies. Individuals living in endemic areas have been shown to generate high-titre binding-inhibitory antibodies against PvDBPII that effectively block DARC binding by various PvDBPII alleles. Importantly, the development of such binding-inhibitory antibodies is correlated with resistance to *P. vivax* infection (18). These findings support the development of a subunit vaccine based on PvDBPII to protect against *P. vivax* malaria, both immunologically and mechanistically (10).

For production of recombinant PvDBPII, codon optimised synthetic gene encoding PvDBPII (M.W ~39kDa) was cloned in pET28a (+) vector and transformed into *E. coli* strain BLR(DE3) pLysS. PvDBPII is expressed as inclusion bodies. Recombinant PvDBPII is denatured, refolded, purified to homogeneity, filter sterilized and stored at -70 °C or below.

Matrix-M™ in clinical studies (also known as 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*™* VAC079 Clinical Trial Protocol, V 6.0, 4 th March 2021, University of Oxford Page 30 of 108 Matrix-M1*™* (MM) is a 40nm-sized complex containing the adjuvant-active saponins obtained by chromatographic fractionation of extracts of the bark of the Quillaja saponaria tree, phospholipid and cholesterol. Quillaja saponins are triterpene glycosides, with molecular weights of the different saponins ranging from 1,800 – 2,000 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 100,000 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 developed in human vaccine clinical trials addressing multiple target antigens. 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 from the same saponin source, 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^{TM} or Matrix-M2^{TM}), has been administered to 37734 individuals, of which 37197 have received Matrix-M1^{TM}, in a total of 29 clinical trials in the US, UK, Europe, Australia and Africa. These trials include assessment of recombinant malaria candidate vaccines, the most advanced of which is the malaria/ HBsAg fusion moiety R21, adjuvanted with Matrix-M^{TM}, within six Phase I and Phase I/II trials conducted in UK, East Africa and West Africa. 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^{TM} 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. The majority of these symptoms are of mild intensity, and they are transient.

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.

Recombinant PvDBPII formulated in Matrix-M adjuvant was assessed in an open-label Phase I/IIa clinical trial (VAC079) in healthy adults living in the UK for safety, immunogenicity and efficacy (Clinicaltrials.gov identifier: NCT04201431) (Ref). The PvDBPII/Matrix M vaccine was administered intramuscularly to 16 healthy malaria-naïve volunteers at a dose of 50 µg PvDBPII protein mixed with 50 µg Matrix-M adjuvant (PvDBPII/Matrix-M). PvDBPII/Matrix-M was administered monthly (0, 1, 2 months) or in a delayed dosing regimen (0, 1, 14 months). The delayed regimen was due to trial halts during the Covid-19 pandemic. Volunteers underwent heterologous controlled human malaria infection (CHMI) with blood-stage *P. vivax* parasites (using the PvW1 inoculum) (13) at 2-4 weeks following their last vaccination, alongside unvaccinated controls. PvDBPII/Matrix-M was found to be safe and well tolerated with no safety concerns in malaria naïve adults. Following vaccinations with PvDBPII/M-M, all solicited AEs were mild to moderate in severity, with subsequent doses associated with increased frequency and severity of solicited AEs as compared to the first dose. The most common solicited AEs reported were injection site pain and headache. Incidents of transient lymphopenia of grade 2 severity were observed following vaccinations with PvDBPII/M-M. Unsolicited AEs, deemed at least possibly related to PvDBPII/M-M vaccinations, were of mild to moderate severity and self-limiting.

When given in a delayed dosing regimen (0, 1 month and 14 months), PvDBPII/Matrix-M elicited high antibody responses against PvDBPII and binding inhibition titers. Following heterologous controlled human malaria infection (CHMI) with blood-stage *P. vivax* parasites, PvDBPII/Matrix-M given in the delayed boosting regimen demonstrated significant reduction (>50%) in the mean parasite multiplication rate in the vaccinated group as compared to unvaccinated controls. However, administration of PvDBPII/Matrix-M in a 0, 1 month and 2 months regimen elicited 10 fold lower antibody and binding inhibition titers and did not have any impact on parasite multiplication rate following CHMI with blood-stage *P. vivax* parasites.

Why controlled human challenge trial?

Conventional vaccine development is complex. The average development timeline is between 8 and 18.5 years, estimated costs are $200 million to $900 million, and the probability of success of less than 10% (19, 20). Vaccine clinical trials aim to answer questions related to the immune response, immune correlates of protection, protective efficacy, and target populations. It is expected that most vaccines will fail because of inadequate protective efficacy. Increasing recognition of this critical bottleneck in conventional vaccine development has led to demand for a proof-of-concept clinical trial: **the controlled human challenge trial**.

In July 2014, the World Health Organization (WHO) held a consultation on Clinical Evaluation of Vaccines: regulatory expectations and controlled human challenge trials were considered important

for facilitating vaccine development (21). Controlled human challenge trials are thus a prime example of ways in which clinical development of vaccines can be accelerated by facilitating early rejection of poor vaccine candidates. Controlled human challenge trials in malaria initially raised ethical debate, but have now gained acceptance through demonstrating their potential and the fact that they have been safely performed in >3,000 volunteers all over the world (22).

The development of a safe and reproducible *P. vivax*-controlled challenge model in humans could facilitate clinical development of *P. vivax* vaccines, through selecting efficacious candidates for further clinical testing in more expensive and logistically challenging studies in the field.

P. vivax human challenged study in Thailand

This study is part of the long-term project "Malaria Infection Study in Thailand (MIST)" funded by Wellcome Trust to develop the feasibility of *P. vivax* controlled human challenge trial in an endemic area. The following section provides an overview of the projects in this programme, which comprises multiple studies to be conducted over the next 5 years in the Faculty of Tropical Medicine, Mahidol University, Thailand.

Most vivax malaria challenge studies performed so far have been in non-endemic settings such as in the US, UK, and Australia. However, the findings in those settings may not be extrapolatable to the target population for future vaccine deployment, which is may differ both in terms of immunity to *P. vivax* and genetic background. The best volunteer population in which to test new vaccines is the eventual target population for vaccine deployment. To date, the MIST1 and MIST2 are the only CHMI trials performed in a *P. vivax* endemic population in Asia.

The MIST1 study aimed to assess the safety and feasibility of controlled human *P. vivax* malaria infection following infection by the natural route of delivery – mosquito bite and provided a source of *P. vivax* infected blood to use in future vaccine efficacy trials of a blood- and transmission-stage vaccines. Up to date, there are two set of inocula obtained from MIST study.

MIST2 study was a blood stage inoculum dose finding study using the inocula produced from MIST1) to identify the dose of the inocula to be used in future studies. In addition, MIST2 study will serve as the basis for a challenge model for future *P. vivax* candidate vaccine efficacy studies.

P. vivax human challenge studies conducted in Thailand would allow us to understand the immunological correlates of protection in an endemic setting, thereby informing the development of new *P. vivax* vaccine candidates and rapid testing of the protective efficacy of candidate vaccines in the at-risk population in which they will be deployed. Advancing the development of such methods needs renewed emphasis on understanding the biology, pathogenesis and transmission of *P. vivax*.

The overall objectives of the MIST programme

- **Proof of feasibility and safety** study and providing *P. vivax* **banked infected blood inocula** for future *P. vivax* blood stage human challenge studies.
- Test the protective efficacy of pre-erythrocytic, blood stage and (ultimately) transmission blocking *P. vivax* **vaccine candidates** in the **target population.**
- Characterise correlates of *P. vivax* immune protection in the **target population**.
- Test the efficacy of **new drug candidates** under development.

To achieve these major aims, the programme will compose of 4 to 7 studies. Each subsequent study will be informed by the previous studies in the series and each study will be covered by separate protocols with separate Ethics Committee submissions and approvals.

The MIST3 study (covered by this current protocol):

This MIST3 study is a Phase II clinical trial to primarily assess the safety of the PvDBPII/Matrix-M^{TM} vaccine in healthy volunteers and to establish whether the PvDBPII/Matrix-M^{TM} vaccine can demonstrate a reduced parasite multiplication rate in vaccinated participants compared to control group (no malaria vaccine) using banked blood from MIST1 at an optimum inoculum dose as determined by MIST2.

B2. STUDY OBJECTIVES AND ENDPOINTS

Primary Objectives:

1. To assess the safety and tolerability of the PvDBPII/Matrix-M^{TM} vaccine.
2. To establish whether the PvDBPII/Matrix-M^{TM} vaccine can demonstrate a reduced parasite multiplication rate in vaccinated participants compared to controls (placebo vaccine) in a *P. vivax* blood-stage controlled human malaria infection model

Primary Endpoints:

1. Safety: Data on specific endpoints for safety and reactogenicity will be collected actively and passively. The following parameters will be assessed:
    a. Occurrence of solicited local reactogenicity signs and symptoms in the 7 days following each vaccination
    b. Occurrence of solicited systemic reactogenicity signs and symptoms in the 7 days following the vaccination
    c. Occurrence of unsolicited adverse events in the 28 days following each vaccination
    d. Change from baseline for safety laboratory measures in the 28 days following vaccination
    e. Occurrence of serious adverse events during the whole study duration and feasibility of primary *P. vivax* blood-stage CHMI, as measured by (S)AE occurrences and successful infection (development of detectable persistent parasitaemia by thick blood film +/- clinical symptoms)

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. 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 and withdrawal due to AE(s)/SAE(s) will be described in detail.

The vaccinated participants will be followed for approximately 1 year following their first vaccination

2. Efficacy Endpoints:

Quantitative PCR-derived parasite multiplication rate (PMR) will be the primary efficacy endpoint and a comparison of the endpoint between the participants vaccinated with PvDBPII/Matrix-M^{TM} and control group will constitute the primary analysis for efficacy.

The geometric means of 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 MIST3 Statistical Analysis Plan. The PMR will be calculated using a linear model fitted to log10-transformed qPCR data. The secondary efficacy endpoint will be log10

cumulative parasitaemia by qPCR (area under the curve) for each individual up to the first day on which a participant is treated.

Secondary objectives:

1. To assess the humoral and cellular immunogenicity of the PvDBPII-Matrix-M^{TM} vaccine candidate.
2. To assess immunological readouts for association with a reduced parasite multiplication rate
3. To assess transmission of the parasite from each infected participant to Anopheles mosquitoes

Secondary Endpoints:

1. Immunological Endpoint: 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 region II (PvDBPII) – total IgG, isotypes and avidity; T cell responses to PvDBPII by flow cytometry assays; in vitro functional PvDBPII inhibitory binding assays and in vitro functional PvDBPII growth inhibition assays. Other established and exploratory immunology assays may be carried out, including through collaboration with other specialist laboratories, which may be outside of Thailand. This would involve transfer of anonymized serum/plasma samples. Consent for this will be requested from participants. Other analyses may be detailed in the MIST 3 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. Any other immunological analyses performed will be reported as not pre-specified in the trial protocol.
    2. PMR and anti-PvDBPII antibody responses.
    3. Percentage of mosquitoes infected and the average oocyst number per mosquito after blood feeding.

B3. STUDY SITE(S)

Clinical Therapeutics Unit (FTM CTU, recruiting site), Hospital for Tropical Diseases, Mahidol Vivax Research Unit (MVRU), Medical Entomology Department, Tropical Medicine Diagnostic Reference Laboratory (TMDR), and Malaria Laboratory, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

B4. STUDY TIMELINES:

1. There will be up to 3 months screening process.
2. The duration that individual participants will be in the study is 1 year and 7 months.
3. After completion of the last participant's last visit, there will be an estimated 3 months for database lock and an estimated 3 months to analyse the data.
4. The total duration of this study is approximately 2 year and 3 months starting after EC approvals.

B5. STUDY POPULATION AND SAMPLE

B5.1 Target population

46 healthy Thai adults, aged from 20 to 55 years will be randomized in a 1:1 ratio to the study malaria vaccine or to a 'sham' vaccine normal saline control group (23 participants per study group). All participants who received 3 doses of the malaria vaccine and those in the control group will be challenged at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

B5.2 Subject selection criteria

B5.2.1 Subject/inclusion criteria

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

1. Healthy Thai adults aged 20 to 55 years
2. Minimum educational level of high school or equivalent
3. Red blood cells positive for the Duffy antigen/chemokine receptor (DARC)
4. Women only: Must practice continuous effective contraception for the duration of the study period until 3 months post-challenge.
5. Agreement to refrain from blood donation during the study and for 1 year after the initiation of antimalarial treatment.
6. Willing to be admitted to the Hospital for Tropical Diseases for clinical monitoring as required by the protocol until antimalarial treatment is completed and their symptoms are settling, willing to take a curative antimalarial treatment following CHMI, and willing to reside in Bangkok and its vicinity for 2 months after malarial treatment initiation.
7. Able to read and write in Thai.
8. Provide written informed consent to participate in the trial
9. Answer all questions on the informed consent quiz correctly

10. Completed COVID-19 vaccination with 2 doses of any WHO-approved vaccine

B5.2.2 Subject/exclusion criteria

The participant **MUST NOT** enter the study if any of the following apply:

1. Positive malaria qPCR OR malaria film prior to vaccination and challenge
2. Presence of any medical condition (either physical or psychological) that, in the judgment of the investigator, would place the participant at undue risk (including the history of clinically significant contact dermatitis) or interfere with the results of the study (e.g., underlying cardiac, renal, hepatic or neurological disease; severe malnutrition; congenital defects or febrile condition)
3. Presence of chronic disease or chronic use of medication
4. Prior receipt of other investigational vaccine which is likely to impact the interpretation of the trial data as assessed by the Investigator.
5. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection, asplenia, history of splenectomy, recurrent severe infections, and chronic infection
6. Immunosuppressant medication within the past 6 months preceding enrolment (D0) or plan to use during the study (inhaled and topical steroids are allowed)
7. History of allergic disease or reactions likely to be exacerbated by malaria infection
8. Female participant who is pregnant as evidenced by positive beta-human chorionic gonadotropin (β-HCG) test, or who is lactating or planning pregnancy during the course of the study.
9. Contraindications to the use of antimalarial treatment (e.g., chloroquine, atovaquone/proguanil, or dihydroartemisinin/piperaquine)
10. Use of medications known to have potentially clinically significant interaction with the antimalarial drugs that will be used in this study (chloroquine, atovaquone/proguanil, or dihydroartemisinin/piperaquine)
11. History of cardiac arrhythmia, including clinically relevant bradycardia or Known existing positive family history in both 1st AND 2nd-degree relatives < 50 years old for cardiac disease
12. Family history of congenital QT prolongation or sudden death
13. Any clinical condition, including using medications known to prolong the QT interval or screening electrocardiogram (ECG), demonstrates a QTc interval ≥ 450 ms.
14. Suspected or known history of alcohol abuse or history of drug abuse.
15. Concurrently participating in another clinical study, at any time during the study period
16. Positive hepatitis B surface antigen or seropositive for hepatitis C virus, or HIV
17. Finding on safety laboratory values as defined below:

- Abnormal ALT [>upper normal range]
- Abnormal serum creatinine [>upper normal range]
- Clinically significant abnormalities in corrected calcium and magnesium blood levels
- Haemoglobin < 11 g/dL

18. Blood group Rhesus negative
19. Blood incompatibility to the inoculum
20. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine
21. Any history of anaphylaxis in reaction to vaccinations

Vaccination and re-vaccination exclusion criteria

1. Acute disease at the time of vaccination. (acute disease is defined as the presence of a moderate or severe illness with or without fever).

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

1. Anaphylactic reaction following administration of the vaccine

2. Pregnancy

Exclusion criteria on the day of CHMI

The following constitute absolute contraindications to CHMI:

1. Acute disease, defined as a moderate or severe illness with or without fever
2. Pregnancy
3. Use of systemic antibiotics with known antimalarial activity in the 30 days before challenge (e.g., trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones, and azithromycin)

B5.3 Sample size calculation

This is a randomized, double-blind, single site, Phase II, blood-stage *P. vivax* malaria vaccine trial to assess the safety, immunogenicity and efficacy of the PvDBPII/Matrix-M^{TM} vaccine candidate against blood-stage challenge for the first time in Thailand, utilising a produced cryopreserved inoculum source. Sample sizes for phase II clinical trials are usually small

because safety information continues to be collected in phase II, hence few individuals are expected to be subjected to intervention due to uncertainties in the safety profiles of the interventions. In this study, the sample size determination is based on the efficacy endpoint. A two-sided, two-sample *t*-test with group sample sizes of 18 participants in the PvDBPII/Matrix-MTM vaccine group and 18 participants in the control group achieves at least 80% power to detect at least a 2-fold reduction in the mean parasite multiplication rate (PMR) in the PvDBPII/Matrix-MTM vaccine group compared to the control group. The coefficient of variation on the original scale is 0.8. The significance level (alpha) is 0.050. Thus, assuming a 20% dropout rate, the number of participants in each group undergoing malaria vaccination and or in the control group will be 23, making a total of 46 participants in the study. The sample size calculations were performed in PASS 2020 using the "Tests for Fold Change of Two Means (Log-Normal Data)" procedure.

B5.4 Recruitment Methods

Volunteers will be screened and recruited at the Faculty of Tropical Medicine's Clinical Therapeutics Unit (FTMCTU), Faculty of Tropical Medicine, Mahidol University in Bangkok following Recruitment Process for Healthy Volunteer Standard Operating Procedure (SOP).

In brief, the volunteers may be recruited by use of recruitment materials approved by the ethics committee and distributed or posted in the following places:

- On the MIST website operated by the study group
- In public places with the agreement of the owner / proprietor
- Via presentations (e.g. presentations at lectures or invited seminars)

In addition, we may contact individuals who have participated in previous clinical trials from the FTMCTU's databases. These volunteers will have expressed an interest in receiving information about all future studies in FTMCTU for which they may be eligible.

B5.5 Withdrawal/discontinuation criteria

In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2013) and any other applicable regulations, a participant 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 participant may withdraw/be withdrawn from further study procedures at any time in the interests of the participant'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).

- Participant non-compliance with study requirements.
- An AE which requires discontinuation of the study involvement or which results in inability to continue to comply with study procedures.

The medical monitors may recommend withdrawal of participants.

The reason for withdrawal from further study procedures will be recorded in the Case Record Form (CRF). If a participant withdraws after having completed a course of antimalarials, as much long-term safety data collection as possible, including procedures, such as safety bloods, will continue to be collected, with the agreement of the participant.

If a participant withdraws from the study after challenge but before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of antimalarial therapy must be completed with standard chloroquine treatment. The importance of this will be emphasized to participants at screening. If a participant refuses to take antimalarial therapy after malaria diagnosis, a rapid assessment of mental state and capacity will be undertaken, with the involvement of psychiatric and infectious diseases specialists. If necessary and if in accordance with the law in Thailand, the participant may be detained for appropriate medical management.

If a participant withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the participant 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. Participants are free to request that their blood samples be destroyed anytime during or after the study.

In all cases of participant withdrawal, except those of complete consent withdrawal, safety data collection will continue if participants have already undergone challenge process.

Whenever possible attempt will be made to replace study participant(s) who withdrawn from the study.

B5.6 Multi-site research

- Not applicable

B6. RESEARCH METHODOLOGY

B6.1 Details of study design

Summary of trial design

Phase II Randomized double-blind controlled study with CHMI, designed to assess the safety, immunogenicity, and protective efficacy of PvDBPII/Matrix-M™ vaccine using RTPCR as the primary diagnostic.

Overview

This is a Phase II randomized double-blinded controlled single-centre *Plasmodium vivax* blood-stage CHMI trial to assess the safety, immunogenicity and efficacy of the candidate malaria vaccine PvDBPII/Matrix-M™. Healthy, Thai adults aged from 20 to 55 years will be recruited and randomized at the Faculty of Tropical Medicine, Mahidol University in Bangkok.

Study group: Up to 23 healthy adults aged from 20 to 55 years will be recruited. These participants will receive three doses of the PvDBPII/Matrix-M™ vaccine (each dose consisting of 50 µg of vaccine and 50 µg of Matrix M) intramuscularly at 0, 1 and 7 months.

Between three to four weeks post-boost (3rd vaccination), all participants who receive 3 doses of PvDBPII/Matrix-M vaccine will undergo *P. vivax* blood-stage CHMI, induced by injection of *P. vivax*-infected erythrocytes.

Control group: Up to 23 healthy adults aged from 20 to 55 years will be recruited.

These participants will receive three doses of placebo vaccine, at a volume identical to that of the PvDBPII/Matrix-M™ vaccine, intramuscularly at 0, 1 and 7 months.

Between three to four weeks post-boost (3rd vaccination), all participants who receive 3 doses of placebo vaccine will undergo *P. vivax* blood-stage CHMI, induced by injection of *P. vivax*-infected erythrocytes.

All participants 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 participants meet criteria for antimalarial treatment or until 21 days after challenge, when treatment will be started empirically.

Therapy will be with a standard course of chloroquine where not contraindicated. As infection will be induced via intravenous injection of blood-stage parasites, there will be no liver-stage infection and no hypnozoite formation, thereby eliminating the need for radical cure with primaquine therapy. Follow-up at study site will be up to 1 year after antimalarial treatment initiated.

Investigational Products

PvDBPII Vaccine

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. The vial is stored at -70ºC or below. Recombinant PvDBPII protein will be formulated at bedside with adjuvant Matrix-M™ just prior to administration in the trial. Further details relating to batch release and manufacturing can be found in the PvDBPII IMPD.

Storage of PvDBPII Vaccine

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™

Matrix M1 is manufactured under Good Manufacturing Practice conditions by Novavax AB (Uppsala, Sweden). Matrix-M1™ will be supplied to the clinical site by Novavax 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 Matrix-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 CTU, Faculty of Tropical Medicine. Further details relating to batch release and manufacturing can be found in the Matrix M1 IMPD.

Storage of Matrix-M™

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.

Administration of Vaccine

Both vaccines (malaria and sham) will be administered intramuscularly in the deltoid muscle of the arm, according to SOP Vaccination. The syringes containing the vaccine or 0.9% saline will be prepared by the study pharmacist and labelled with the participant's study numbers in accordance with the randomization list. 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, the study vaccine and adjuvant will be allowed to thaw to room temperature and will be administered within 1 hour. For each immunization, one vial of PvDBPII will be used per participant. 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^{TM} will be administered to all participants assigned to the vaccine group. Matrix-M1^{TM} and PvDBPII will be mixed at the bedside immediately prior to administration according to vaccine administration SOP. Any vaccine not administered within the time frame will be destroyed.

PvDBPII antigen (200 micrograms per mL)	Matrix-M^{TM} (375 micrograms per mL)	Volume immunized
0.39 mL	0.21 mL	0.38 mL

i. 0.39 mL of the thawed antigen will be mixed with 0.21 mL of Matrix M^{TM} adjuvant.
ii. Mix by ten gentle manual inversions to prepare the formulation.
iii. From this reconstituted solution, 0.38 mL will be withdrawn for intramuscular administration to the participants.

The control 'sham' vaccine syringes will contain 0.38 mL of 0.9% saline.

Vaccine calculation

	Vaccine (PvDBPII) 0.5 mL per vial	Matrix-M^{TM} 0.75 mL per vial
1 dose	1 vial	1 vial
3 doses	3 vials	3 vials
3 doses x 23 participants	69 vials	69 vials

B6.2 Details of procedures for specimen/data collection

B6.2.1 Screening and eligibility assessment

The screening will aim to recruit 46 healthy volunteers (23 volunteers per study group).

Screening visits may take place up to 30 days prior to enrolment. Informed consent will be conducted at screening. If consent is given, the screening procedures will be undertaken, including testing for Duffy antigen/receptor for chemokine (DARC) positivity, blood typing, blood diseases, CBC, blood biochemistry, blood-borne infections, malaria diagnosis, malaria exposure, malaria immunology, and serum pregnancy test. The full list of laboratory tests and the schedules of procedures is listed in Table 4.

Abnormal clinical findings from the medial history, physical examination, or laboratory tests at any point in the study will be assessed according to the scales in APPENDIX C: Safety terms and administrative information. If a test is deemed clinically significant it may be repeated to ensure it is not a single or spurious occurrence. If an abnormal finding is deemed to be clinically significant, the participant will be informed and appropriate medical care arranged with the permission of the participant.

Cross-matching (Gel Coombs Card method) will be performed to check blood compatibility between participant and the blood inoculum.

Exclusion of the participant from enrollment in the trial or withdrawal of a participant from the trial will be at the discretion of the Investigators.

B6.2.2 Enrollment and first vaccination with PvDBPII-Matrix-M^{TM} (D_0) and days 3 (D_3)

The eligibility of the participant 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 participant to be enrolled into the study (study vaccine group or control group).

At the day 0 visit, any new medical issues or symptoms that have arisen will be assessed.

Physical observations/examination, urinary β-HCG test in female participants and venipuncture for immunological assays, Immune cells & cytokines profile and safety bloods will be undertaken according to Table 5. The inclusion and exclusion criteria for the study will be reviewed. If the participant remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix-M^{TM} vaccine or 0.9% saline will be administered as described in section 6.1.

Participants will be asked to record all AEs via the diary card and bring it to the upcoming visit.

At the day 3 visit (post-vaccination), blood will be taken for immunological assays.

B6.2.3 Reviews post-vaccination on days 7 (D_7) and days 14 (D_{14})

On these subsequent visits, the participants will be assessed for local and systemic adverse events, using a diary card, well-being check list, physical examination, and blood tests at the time points indicated in the schedule of attendances (Table 5). Blood will also be taken for immunological assays and Immune cells & cytokines profile on days 7 and 14. On the Day 7 visit participants will be asked directly about foreseeable local and systemic AEs for the 7 days post vaccination (solicited AEs).

B6.2.4 2nd Vaccination with PvDBPII-Matrix-M™ (D_{28})

This visit will include Day 28 follow up for the first vaccination and administration of the second vaccine. Any new medical issues or symptoms that have arisen will be assessed. Physical observations/examination, urinary β-HCG test in female participants and venipuncture for immunological assays, Immune cells & cytokines profile, CBC, and blood biochemistry will be undertaken according to Table 5. The inclusion and exclusion criteria for the study will be reviewed. If the participant remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix-M™ vaccine or 0.9% saline will be administered intramuscularly in the opposite arm to that used for the first vaccination and as described in section 6.1.

B6.2.5 Reviews on 7 days post 2nd Vaccination (D_{35}), days 42 (D_{42}), and days 56 (D_{56})

On these subsequent visits, the participants will be assessed for local and systemic adverse events, using a diary card, well-being check list, physical examination, and blood tests at the time points indicated in the schedule of attendances (Table 5). Blood will also be taken for immunological assays on days 35, 42, and 56 and Immune cells & cytokines profile on days 35 and 42. On the Day 35 visit participants will be asked directly about foreseeable local and systemic AEs for the 7 days post the second vaccination (solicited AEs).

B6.2.6 3rd Vaccination with PvDBPII-Matrix-M™ (D_{210}/ Month 7)

The 3rd vaccination will occur approximately 6 months after the second vaccination.

Any new medical issues or symptoms that have arisen will be assessed. Physical observations/examination, urinary β-HCG test in female participants and venipuncture for immunological assays and safety bloods will be undertaken according to Table 5. The inclusion and exclusion criteria for the study will be reviewed. If the participant remains eligible and there are no contraindications to vaccination, the PvDBPII-Matrix-M™ vaccine will be administered intramuscularly in the opposite arm to that used for the second vaccination and as described in sections 6.1.

At this visit, laboratory tests such as blood biochemistry, CBC, immunological assays, Immune cells & cytokines profile and urine pregnancy test (women only) will be done as described in Table 5.

B6.2.7 Reviews on 7 days post 3rd Vaccination (D_{217}) and days 224 (D_{224})

On these subsequent visits, the participants will be assessed for local and systemic adverse events, using a diary card, well-being check list, physical examination and blood tests at the time points indicated in the schedule of attendances (Table 5). Blood will also be taken for

immunological assays and Immune cells & cytokines profile on days 217 and 224. On the Day 217 participants will be asked directly about foreseeable local and systemic AEs for the 7 days post the third vaccination (solicited AEs).

Exclusion of the participant from inoculation or withdrawal of a participant from the trial will be at the discretion of the Investigators.

B6.2.8 Day before CHMI (Admission day; C_{-1})

About 3-6 weeks after the 3rd vaccination, participants will attend to be admitted to the study facility for CHMI. Any new medical issues or symptoms that have arisen will be assessed. All participants who are eligible for the inoculation will have physical examination/observations performed during this visit. Serum β-HCG (woman), malaria diagnosis, immunological assays, Immune cells & cytokines profile, complete blood count (CBC), and biochemistry will be tested during this visit according to Table 6. Blood for malaria immunology will be taken for baseline information only and the results will not be used as exclusion criteria. Results of the other blood tests taken at this visit must be available and reviewed prior to challenge.

B6.2.9 Day of CHMI (C_0)

All available participants who have not dropped out at this stage and are eligible will be challenged.

CHMI will be administered according to local Standard Operating Procedure (SOP). This will include:

- Well-being checklist and examination of the injection site and any body systems felt to be necessary by the Investigator and verification of eligibility / contraindications.
- Physical observations (including respiratory rate, pulse rate, blood pressure and temperature), with recordings to be repeated if this is longer than 60 minutes before administration of the parasitized erythrocyte inoculum.
- Intravenous access via cannulation in a forearm vein, flushed with normal saline.
- For each participant, the inoculum must be injected within 4 hours of the inoculum being thawed, followed by a further normal saline flush. “see B6.2.14 for details of the inoculum”.
- Physical observations will be repeated at 15 minutes post-administration of inoculum and again at 1 hour, in order to assess for immediate adverse reactions, with regular visual observation throughout, in line with The British Society for Haematology Guidelines for the administration of blood products (23). If the participant shows signs or symptoms of a transfusion reaction, observations will be repeated and the participant will be medically reviewed by a physician.

- Participants 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.

B6.2.10 Days 1 to day of reaching treatment criteria ($D_{\text{reach treatment criteria}}$)

This section describes the clinical procedures for evaluating study participants and management after challenge.

B6.2.10.1 Day 1 post challenge to malaria qPCR show positive (D_{qPCR+})

The participants will be assessed once daily from day 1 post challenge until malaria qPCR becomes positive ($D_{qPCR}+$). The assessment includes a clinical well-being check, and the participants will be questioned whether they have experienced any malaria-related symptoms (such as fever, chills, sweating, malaise). The once daily general vital signs and physical examination will be done. Blood will be drawn once daily for monitoring blood parasitaemia (malaria blood film, qPCR, and gametocyte qPCR).CBC will be performed on day C_4 and D_{qPCR+}. The complete list of laboratory tests, immunological assays, and physical observations/examination are shown in Table 6.

At Day 1 post challenge, if participants do not have any adverse events from the challenge process, they may be discharged from the hospital and come to CTU for the daily study follow-up visits. The participants will be re-admitted to the hospital at any time if clinically indicated.

To ensure that all participants will receive antimalarial treatment according to the study protocol, participants need to stay near by the study site or convenient accommodation will be provided to participants. In addition, participants will be asked to provide a telephone contact of their relative or neighbour to ensure that the study team can reach participants in case of participant loss to follow-up before they complete antimalarial treatment.

B6.2.10.2 Day after qPCR+ ($Day_{After\ qPCR+}$) to day of reach treatment criteria ($D_{reach\ treatment\ criteria}$)

Once malaria qPCR has been detected or the antimalarial treatment criteria have been met, participants will be admitted to the hospital for close monitoring. The clinical well-being will be checked and blood will be drawn twice daily for monitoring blood parasitaemia (malaria blood film, qPCR, and gametocyte qPCR). For membrane feeding to assess gametocyte transmissibility, blood will be collected once daily after qPCR positive until day discharge. Immunological assays, Immune cells & cytokines profile, urine pregnancy test (women only), CBC, and blood biochemistry will be assessed on $D_{\text{reach treatment criteria}}$

Participants will be assessed after challenge according to the schedule in Table 6. Physical observations will be performed. Venipuncture will be performed as per schedule of attendance (Tables 6).

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.

Full physical examination will be performed if deemed necessary by the Investigators. The severity of symptoms will be assessed using the grading criteria summarised in APPENDIX C: Safety terms and administrative information.

Antimalarial treatment will be instituted when the criteria described below are met (Table 2).

If the antimalarial treatment criteria have not yet been met, the treatment shall be initiated at day 21 after challenge (C_{21}). Participants will be closely monitored until completion of antimalarial therapy, as described above.

B6.2.11 Antimalarial treatment

A study physician will then immediately prescribe antimalarial treatment with chloroquine, in accordance with Table 3. Any side effect from the antimalarials will be rapidly dealt with in a controlled environment. They will continue to have blood films and qPCR once daily until clinically recovered AND two consecutive malaria blood films are negative (completion of the chloroquine treatment course). The participants will then be discharged from the hospital.

If any participant reaches day 21 post-challenge without meeting the treatment criteria, they shall be started on a 3-day course of antimalarial treatment (chloroquine).

If any contraindications to chloroquine are identified, an alternative antimalarial (dihydroartemisinin + piperaquine or Malarone (atovaquone + proguanil)) will be prescribed. If a participant is unable to tolerate an oral antimalarial, the appropriate parenteral antimalarial therapy will be prescribed.

If a participant withdraws/is withdrawn from the study after challenge but before reaching the criteria for antimalaria treatment, then a **complete, appropriate, curative course of antimalarial therapy must be completed.** The importance of this will be emphasized to participants at screening.

Table 2*:* Antimalarial treatment criteria.

Malaria-treatment Start Criteria	• Symptoms suggestive of malaria infection with the presence of blood parasitaemia by blood film microscopy. • Asymptomatic with ≥ 50,000 parasites/mL by blood film microscopy OR • Asymptomatic and no parasitaemia until Day C21 Note: In a case where a participant develops symptoms suggestive of malaria infection but ***negative for malaria (malaria blood film or qPCR)***other causes of symptoms should be considered and investigate and treated as appropriate.

Table 3: Chloroquine treatment dose

Weight/age	Day 1	Day 2	Day 3
	CQ Tab	CQ Tab	CQ Tab
50 kg or more (above 14 years)	4	4	2

Reference: National Malaria Treatment Guideline 2019 (Thailand), CQ 150 mg base/tablet

Supportive treatments and medications

Any malaria symptoms (e.g. fever, nausea, vomiting) will be relieved accordingly. Paracetamol (500 mg orally up to four times a day) and dimenhydrinate (50 mg orally) will be prescribed according to clinical need. Intravenous fluid supplementation may be given following physician decision.

In addition, all concomitant medications used in the trial will be recorded in the medical record and CRF throughout study period.

If the participants meet the criteria for severe vivax malaria (by WHO Guidelines for malaria 13 July 2021) or fail to improve within 48 hours of starting antimalarial therapy, malaria experts in the Faculty of Tropical Medicine will be consulted. The participants will be managed according to National Malaria Treatment Guidelines (Thailand) as well as the Hospital for Tropical Diseases' standing order/ procedures / guidelines for severe malaria.

The Investigators are able to treat any participant for malaria regardless of the blood film microscopy or qPCR result if they are clinically concerned or a participant wishes to withdraw from the study.

B6.2.12 Follow up assessment phase (after discharge)

On the antimalarial treatment end date, the participants will receive full counselling on the signs and symptoms that they should observe and report in the diary card and seek medical advice. A diary card to self-document any abnormal symptoms will be given to them, and the contact channel with the study team will again be emphasized. After discharge from the hospital, the follow-up assessment will be performed at FTMCTU as an out-patient on day 7 post treatment initiation (D_{Rx7}), day C28 (C_{28}), day C56 (C_{56}), day C96 (C_{96}), day C180 (C_{180}), and day C276 (C_{276}). On the follow-up days, diary cards will be reviewed and collected (until day C_{96}), a history taken using the clinical well-being checklist, a physical examination will be performed, and AE(s) assessed. Venepuncture will also be performed to detect malaria parasites by blood film and qPCR, and malaria gametocyte qPCR, immunological assays, malaria immunology, CBC, and biochemistry according to Tables 6.

During this follow up phase, the participants will be contacted fortnightly (biweekly) until C_{96} by email / phone call / other social communications e.g. message (SMS), WhatsApp, Line, to ensure they remain well and asymptomatic. The participant will be asked to and agree to stay in Bangkok until 2 months after antimalarial treatment initiation, and always immediately contact the study team as soon as they observe any abnormal symptoms or would like to contact the team for any other reason.

Table 4: Screening schedule of clinical and study procedures for participants

Event	Screening (within 30 days prior to Day 0)
Inclusion / Exclusion criteria	X
Informed Consent Questionnaire	X
Informed consent	X
Medical History	X
Physical Examination	X
Body weight and height[a]	X
Urinalysis	X
Electrocardiogram	X
Vital signs[b]	X
Blood typing ABO, Rhesus, Crossmatching	X
Duffy antigen/receptor for chemokine (DARC)	X
CBC	X
Haemoglobinopathies and thalassaemia traits*	X
G6PD*	X
Blood biochemistry[c]	X
HIV, HBV, HCV[d]	X
Malaria diagnosis (Malaria antigen – blood film and qPCR)	X
Malaria exposure*	X
Malaria immunology*	X
β-HCG testing (women only)	X (Serum pregnancy test)

Remark:

[a] Height will be measured only at screening visit.

[b] Vital signs include blood pressure, pulse rate, respiratory rate and temperature.

[c] Biochemistry including electrolytes (sodium, potassium, chloride, bicarbonate, calcium and magnesium), blood urea nitrogen (BUN), creatinine (Cr), eGFR, liver function tests (LFTs), and FBS

[d] HIV, HBV, and HCV will be tested for antibodies. HBs serological profile includes HBsAg, anti-HBc, and anti-HBs.

*This test will be done for data interpretation purpose. It will not be used as the inclusion/exclusion criteria.

Table 5: Schedule of Clinic Attendances during vaccination

Event	Vaccination Phase										
	(V1)				(V2)				(V3)		
Timeline	D_0	D_3	D_7	D_{14}	D_{28}	D_{35} (7 ds after vac)	D_{42} (14 ds after vac)	D_{56} (28 ds after vac)	D_{210} (Month 7)	D_{217} (7 ds after vac)	D_{224} (14 ds after vac)
Window (days)	0	0	±1	±3	±3	±1	±3	±3	±3	±1	±3
Inclusion/exclusion criteria	X				X				X		
Medical history (well-being check list)	X		X		X	X			X	X	
Body weight	X				X				X		
Physical observation/examination	X		X		X	X			X	X	
Urine pregnancy test (women only)	X				X				X		
Vital signs[a]	X	X	X	X	X	X	X	X	X	X	X
AE/SAE	X	X	X	X	X	X	X	X	X	X	X
Diary card			X			X				X	
Vaccination	X				X				X		
Haematology (CBC)	X		X		X	X			X	X	
Biochemistry[b]	X		X		X	X			X	X	
HIV, HBV, HCV[c]											X[#]
Malaria diagnosis (Malaria antigen – blood film and qPCR)	X										X[#]
Immunological assays ELISAs & GIA[d]	X			X	X	X	X	X	X	X	X
Immunological assays: Humoral and cellular immune responses[e]	X	X	X	X		X	X		X	X	
Immune cells & cytokines profile (Phenotypic profile)	X		X	X	X	X	X		X	X	X

Remark:

[a] Vital signs include blood pressure, pulse rate, respiratory rate and temperature.

[b] Biochemistry including electrolytes (sodium, potassium, chloride, bicarbonate, calcium and magnesium), blood urea nitrogen (BUN), creatinine (Cr), eGFR, liver function tests (LFTs)

[c] HIV, HBV, and HCV will be tested for antibodies. HBs serological profile includes HBsAg, anti-HBc, and anti-HBs.

[d] Test at Oxford

[e] Test at Pasteur
[#] Check for Pre-challenge criteria

Table 6: Schedule of Clinic Attendances since challenge **(including admission C_{-1})**

Event	Admission				Admission		Follow-up Phase					
Timeline	C_{-1}	C_0*	C_1	$C_2 - D_{qPCR+}$	$D_{after\ qPCR+} - D$ reach treatment criteria	$D_{Start\ CQ} - D$ discharge	D_{Rx7}	C_{28}	C_{56}	C_{96}	C_{180}	C_{276}
Window (days)	0	0	0	0	0	0	±2	±7	±7	±7	±14	±14
Inclusion/exclusion criteria	X											
Medical history (well-being check list)	X	X	X (once daily)	X (once daily)	X (Twice daily)	X (once daily)	X	X	X	X	X	X
Body weight	X	X			X ($D_{reach\ treatment}$ criteria)		X	X	X	X	X	X
Physical observation/ examination	X	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test (women only)	X[g]				X (before treatment)							
Vital signs[a]	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE[b]	X	X	X	X	X	X	X	X	X	X	X	X
Blood stage challenge		X										
Treatment for Malaria[c]						X						
Diary card			X			$D_{complete}$ antimalarial treatment	X	X	X	X		
Malaria antigen (Blood Film and qPCR)	X		X (daily)	X (daily)	X (twice daily)	X (daily)	X	X	X	X	X	X
Malaria Gametocyte qPCR			X (daily)	X (daily)	X (twice daily)	X (daily)	X	X	X	X	X	X
Haematology (CBC)	X			C_4, D_{qPCR+}	X ($D_{reach\ treatment}$ criteria)		X	X	X	X	X	X
Crossmatching	X											

Event	Admission			Admission		Follow-up Phase					
Timeline	C_{-1}	C_0*	C_1	C_2 – D $qPCR^+$	$D_{after\ qPCR}$+ – D reach treatment criteria	$D_{Start\ CQ}$ – D discharge	D_{Rx7}	C_{28}	C_{56}	C_{96}	C_{180}	C_{276}
Window (days)	0	0	0	0	0	0	±2	±7	±7	±7	±14	±14
Biochemistry[d]	X			D_{qPCR+}	X ($D_{reach\ treatment\ criteria}$)	D after complete antimalarial treatment	X	X	X	X		X
Immunological assays ELISAs & GIA[e]	X			X (C+7, C+14, $D_{reach\ treatment\ criteria}$)				X	X			X
Immunological assays: Humoral and cellular immune responses[f]	X			X (C+14, C+21)				X	X	X		
Immune cells & cytokines profile (Phenotypic profile)	X			X (C+21)								
MFA[j]					X (once daily)	X (once daily)						
COVID-19 serology test	X				X[h]							
CMV and EBV serology	X				X[i] (C+14)					X		
Malaria immunology	X			C_4, D_{qPCR+}	X (D reach treatment criteria)		X	X	X	X	X	X

Remark:

[a] Vital signs include blood pressure, pulse rate, respiratory rate and body temperature.

[b] AEs/SAEs will be collected until 1 year after antimalarial initiation.

[c] This treatment period could occur on any day between C_6 and C_{21}. Blood draws will continue as per this Table for the relevant study day, up until the time-point of treatment. The treatment will be done on C_{21} if no criteria meet.

[d] Biochemistry including electrolytes (sodium, potassium, chloride, bicarbonate), blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs)

[e] Test at Oxford

[f] Test at Pasteur

[g] Serum β-HCG

[h] COVID-19 serology test will be done at day discharge.

[i] EBV and CMV serology will be done at 2-4 weeks and at 3 months after the inoculation. EBV and CMV serology including EBV VCA (IgM)/IgG antibody, anti EBNA and EA complex antibody, and CMV IgM/IgG antibody

[j] Membrane Feeding Assay (MFA) will be conducted according to SOP Dissection of Anopheles mosquito's midgut to determine infection with malaria parasite and SOP Membrane feeding assay

- * In case volunteer lost to follow-up after 3rd vaccination and then return, the maximum time allowancw between 3rd vaccination and challenge duration is 6 weeks but pre-challenge lab tests need to be repeated as appropriate at investigators discretion.

Table 7: Blood collection during study period

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
Screening	6	Blood typing, crossmatching	2	Malaria antigen (Blood film, qPCR)	2	FBS	31	31
		Blood biochemistry	6	Duffy blood group				
		HIV		CBC+Thalassemia				
		HBs profile, anti-HCV, serum HCG		Hemoglobinophathies				
			1	Malaria exposure				
			12	Malaria immunology				
			2	G6PD FST				
Vaccination D0 (V1 day0)	5	Biochemistry	2	CBC			37	68
	10	Immunological assays (humoral &cellular responses)	2	Malaria antigen				
	10	Immunological functional assays (ELISA&GIA)	6	Immunological assays (humoral &cellular responses)				

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
			2	Immune cells & cytokines profile (Phenotypic profile)				
Vaccination D3 (V1 day3)			6	Immunological assays (humoral &cellular responses)			6	74
Vaccination D7 (V1 day7)	5	Biochemistry	6	Immunological assays (humoral &cellular responses)			15	89
			2	Immune cells & cytokines profile (Phenotypic profile)				
			2	CBC				
Vaccination D14 (V1 day14)	4	Immunological functional assays (ELISA&GIA)	2	Immune cells & cytokines profile (Phenotypic profile)			8	97
	2	Immunological assays (humoral &cellular responses)						
Vaccination D28 (V2 day0)	5	Biochemistry	2	CBC			13	110
	4	Immunological functional assays (ELISA&GIA)	2					

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
				Immune cells & cytokines profile (Phenotypic profile)				
Vaccination D35 (V2 day 7)	5	Biochemistry	2	CBC			19	129
	4	Immunological functional assays (ELISA&GIA)	6	Immunological assays (humoral &cellular responses)				
			2	Immune cells & cytokines profile (Phenotypic profile)				
Vaccination D42 (V2 day 14)	5	Immunological assays (humoral &cellular responses)	2	Immune cells & cytokines profile (Phenotypic profile)			11	140
	4	Immunological functional assays (ELISA&GIA)						
Vaccination D56 (V2 day 28)	4	Immunological functional assays (ELISA&GIA)					4	144
Vaccination D210 (V3 day0)	5	Biochemistry	2	CBC			21	165
	2	Immunological assays (humoral &cellular responses)	6	Immunological assays (humoral &cellular responses)				

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
	4	Immunological functional assays (ELISA&GIA)	2	Immune cells & cytokines profile (Phenotypic profile)				
Vaccination D217 (V3 day7)	5	Biochemistry	2	CBC			19	184
	4	Immunological functional assays (ELISA&GIA)	6	Immunological assays (humoral &cellular responses)				
			2	Immune cells & cytokines profile (Phenotypic profile)				
Vaccination D224 (V3 day14)	2	HIV HBV HCV	2	Immune cells & cytokines profile (Phenotypic profile)			16	200
	10	Immunological functional assays (ELISA&GIA)	2	Malaria antigen (blood film, qPCR)				
Admission day (C-1)	6	Crossmatching	2	CBC			80	280
		Biochemistry, serum HCG	3	Malaria antigen (blood film, qPCR)				
	1	COVID-19 serology	2	Immune cells & cytokines profile (Phenotypic profile)				

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	**Test**	**Volume**	**Test**	**Volume**	**Test**		
	4	CMV&EBV serology	30	mAB production				
	10	Immunological assays (humoral &cellular responses)	12	Malaria immunology				
	10	Immunological functional assays (ELISA&GIA)						
Admission day C1-DqPCR+ (C1-~C8)	5	Biochemistry (DqPCR+)	24	Malaria (blood film, qPCR, qGAM) (once daily)			61	341
	4	Immunological functional assays (ELISA&GIA) (C7)	4	CBC (C4, DqPCR+)				
			24	Malaria immunology (C4, DqPCR+)				
D afterqPCR+-D reach treatment (~C9-~C16)	5	Biochemistry (D reach treatment)	48	Malaria (blood film, qPCR, qGAM) (twice daily)			89	430
	8	Immunological functional assays (ELISA&GIA) (C14,D reach treatment)	8	Membrane feeding assay (once daily)				
	4	CMV&EBV serology (C14)	2	CBC (D reach treatment)				
	2	Immunological assays (humoral &cellular responses) (C14)	12	Malaria immunology (D reach treatment)				

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
D after start CQ- D discharge (~C17-~C21)	5	Biochemistry (D after complete treatment)	6	Immunological assays (humoral &cellular responses) (C21)			34	464
	1	COVID-19 serology (D discharge)	2	Immune cells & cytokines profile (Phenotypic profile) (C21)				
			15	Malaria (blood film, qPCR, qGAM) (once daily)				
			5	Membrane feeding assay (once daily)				
DRx7 (~C22)	5	Biochemistry	3	Malaria (blood film, qPCR, qGAM) (once daily)			22	486
			12	Malaria immunology				
			2	CBC				
C28	5	Biochemistry	3	Malaria (blood film, qPCR, qGAM) (once daily)			24	510
	2	Immunological assays (humoral &cellular responses)	12	Malaria immunology				

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
			2	CBC				
C56	5	Biochemistry	3	Malaria (blood film, qPCR, qGAM) (once daily)			28	538
	4	Immunological functional assays (ELISA&GIA)	12	Malaria immunology				
	2	Immunological assays (humoral &cellular responses)	2	CBC				
C96	4	CMV&EBV serology	12	Malaria immunology			32	570
	5	Biochemistry	3	Malaria (blood film, qPCR, qGAM) (once daily)				
	2	Immunological assays (humoral &cellular responses)	2	CBC				
	4	Immunological functional assays (ELISA&GIA)						
C180			3	Malaria (blood film, qPCR, qGAM) (once daily)			17	587
			12	Malaria immunology				

Phase	Clot blood		EDTA blood/ WB Tempus		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume	Test	Volume	Test	Volume	Test		
			2	CBC				
C276	5	Biochemistry	3	Malaria (blood film, qPCR, qGAM) (once daily)			26	613
	4	Immunological functional assays (ELISA&GIA)	12	Malaria immunology				
			2	CBC				

B6.2.13 Details of study clinical procedures/laboratory tests

Procedures will be performed at the time points indicated in the schedule of procedures (Tables 4 - 6). Additional procedures or laboratory tests may be performed, at the discretion of the investigators if clinically necessary. Study procedures will be monitored by the MORU Clinical Trials Support Group.

Clinical procedures

Body Weight and height

Body Weight and height will be measured at the screening (Table 4) and body weight will be measured as indicated in the Table 5 and 6.

Vital signs

Pulse rate (PR), blood pressure (BP), respiratory rate (RR) and body temperature (BT) will be measured at the time points indicated in the schedule of procedures (Tables 4 and 5).

Urinalysis

Urine will be tested for the presence of clinically significant proteinuria, glucosuria or haematuria at the screening visit.

Electrocardiogram

An electrocardiogram will be performed at the screening visit.

Laboratory procedures

The Tropical Medicine Diagnostic Reference Laboratory (TMDR) will be the central reception point for all blood samples collected from FTMCTU, and will follow a sample management system designed by the MORU Sample Management Centre. TMDR will aliquot diagnostic and research samples and be responsible for transfer to designated laboratories for testing and retrieve the result generated from the contracted laboratories.

Samples will also be sent to collaborating laboratories within and outside Thailand for immunomonitoring and/or harmonisation of key immunological assays. There are four collaborating laboratories:

(1) Ehime University, Matsuyama, Japan
(2) Kenya Medical Research Institute (KEMRI), Kenya
(3) Institute Pasteur, Paris

(4) University of Oxford, UK

The AlphaScreen assay will be performed at Ehime University to quantify malaria antigens-specific antibodies (up to 300 *P. falciparum* antigens and 300 *P. vivax* antigens). A seropositive cut-off will be set as half the lowest non-negative value of the assayed samples. Positive malaria history refers to a participant who previously had a malaria infection leading to antibodies in their serum which are reactive to more than 10% of the malaria antigens in the library.

B6.2.14 Challenge infection performed in the trial participants

B6.2.14.1 Description and administration of the cryopreserved P. vivax infected erythrocytes

The total volume of the diluted cryopreserved parasite that will be injected intravenously into each participant is 5 mL.

B6.2.14.2 Source and preparation of the cryopreserved inoculum

The cryopreserved *P. vivax* parasitized red blood cells that will be used were produced *by investigator team* in *December* 20*20*, as part of "A clinical study to assess the feasibility of a controlled human *Plasmodium vivax* malaria infection model through experimental sporozoite infection in Thai adults" ('MIST 1 study', certificate number 2020-038-01, approved by the FTM-EC #TMEC19-067; OxTREC Ref 43-19. The information is as follows.

Cryopreservation and storage of blood bank

P. vivax infected blood processing was conducted within fumigated microbiological safety cabinets. First, the red cells were separated from plasma by centrifugation of the leukodepleted blood before mixing with GMP grade 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 1.5 mL cryovials and frozen at -80ºC for one night before transferred to a dedicated liquid nitrogen tank. Confirmation of parasitic density within the blood collected for cryopreservation was performed via microscopy and quantitative PCR on the leukodepleted packed blood samples. Thick film microscopy demonstrated 6 asexual parasites per 1 µL leukodepleted packed blood for Donor 1, and 13 asexual parasites per 1 µL leukodepleted packed blood for Donor 2. Quantitative PCR confirmed the presence of 155,400 genome copies/mL in Donor 1, and 988,600 genome copies/mL in Donor 2.

Sterility and screening of blood-borne and vector-borne infections of cryopreserved blood bank

Sterility and screening for blood-borne and vector-borne infections of the cryopreserved blood bank were performed to ensure the safety of the cryopreserved blood bank. Real time PCR for HIV-1, HIV-2, HBV, HCV, Dengue, chikungunya, zika, Japanese encephalitis, CMV and EBV, serology for HTLV-1, HTLV-2 and syphilis (TPHA), serology for filaria, endotoxin and ELISA for mycoplasma were tested from plasma derived directly from blood bank. Blood collected for cryopreservation was sent to FTM hospital for testing the bacterial contamination using hemoculture technique. All tested results were negative for both donors.

B6.2.14.3 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 local standard operating procedure. The processing of the inoculum will be carried out in MVRU's dedicated biosafety level II laboratory at the Hospital for Tropical Diseases, Faculty of Tropical medicine. Sample manipulations will be performed within a safety cabinet that has been fumigated, sterilized and dedicated for this purpose. Deglycerolizing will be performed by sequentially adding 0.1 volume of 12%, 10 volume of 1.6% and 10 volume of 0.9% saline solution. The inoculum will be reconstituted in 0.9% saline, to a total volume of 5 mL***. Further dilution of the incoculum will be undertaken*** based on the result***s of the*** MIST***2, which at the time of writing the first version of this protocol are still pending.*** Five milliliters of the diluted inoculum will be drawn in 5 mL syringe and closed with sterile cap. The inoculum will be kept in the warm transport box until inoculation in the participants. The inoculum must be injected ***intravenously into the participant*** within 4 hours of the inoculum being thawed.

B6.2.14.4 Administering the study agent to the participants:

The inoculation will take place at the Hospital for Tropical Diseases. The inoculum will be administered by injection into an indwelling intravenous cannula.

The challenge steps:

The participant(s) will be hospitalized a night before the inoculation day (C_0) to ensure their safety /eligibility and to avoid the traffic burden.

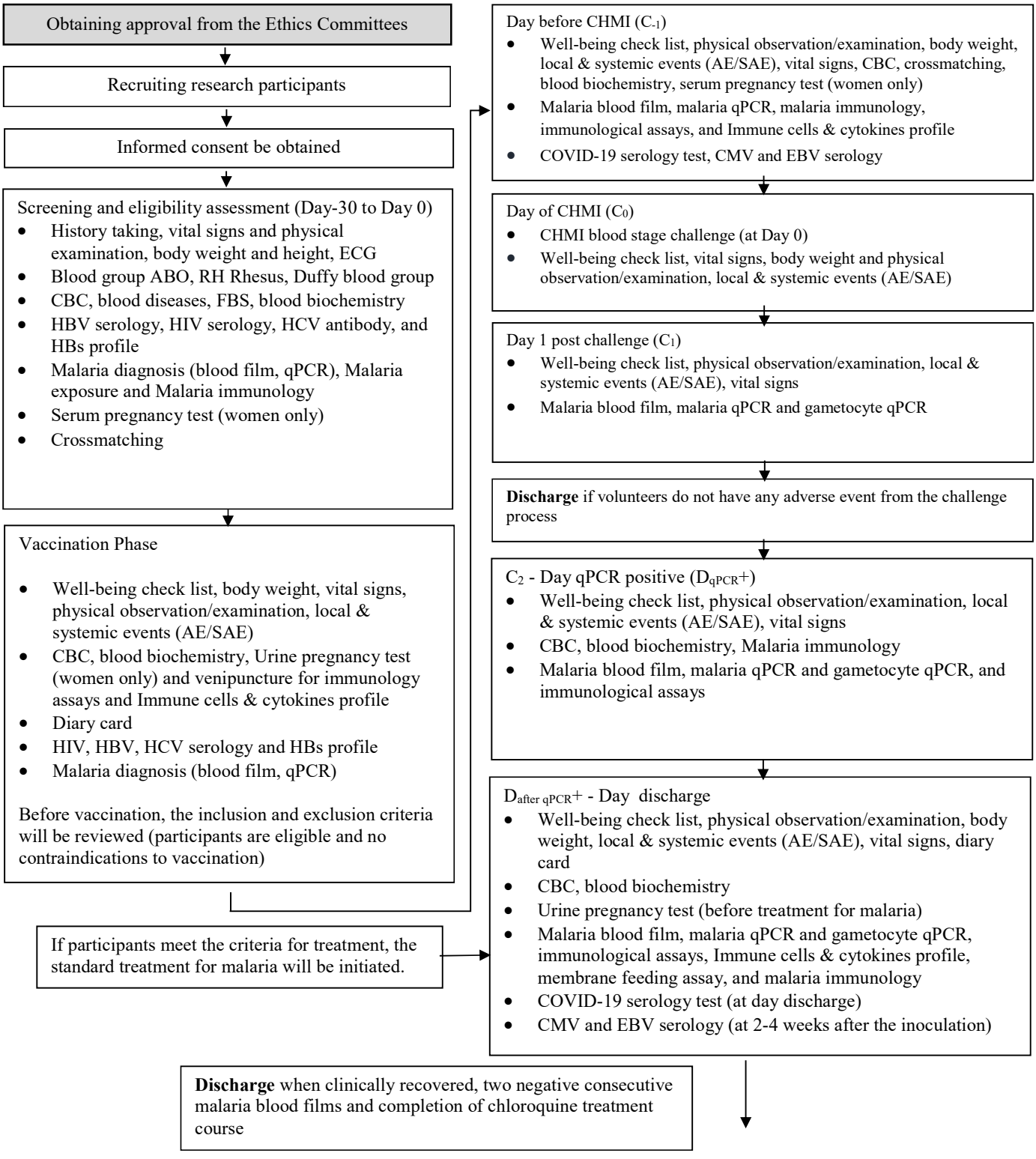
Prior to challenge, all participants are briefed. The arms will be cleaned by using 70% alcohol according to the Inoculum Administration Work Instruction (WI).

The inoculum will be transported directly from the MVRU clean room lab at 9th floor in a warm box to the admission facility within the Hospital for Tropical Diseases where the injection of the inoculum being take place.

All groups will receive a total of 5 ml of inoculum diluted with 0.9% normal saline. This dilution selected will be based on the optimal dose finding result of the MIST2 study (currently still pending).

The reconstituted inoculum will be injected via an indwelling cannula, followed by a saline flush.

B6.3 Schematic diagram of study design, procedures and stages, step-by-step



During follow up phase volunteer will be contacted fortnight until C_{96} by email/ phone call/ other social communications e.g. message (SMS), WhatsApp, Line to ensure they remain well and asymptomatic.

Follow up phase

- Well-being check list, and physical observation/examination, vital signs body weight, local & systemic events (AE/SAE), diary card
- CBC and blood biochemistry
- Malaria blood film, malaria qPCR and gametocyte PCR, immunological assays, and malaria immunology

B6.4 Specimen and data management

The Tropical Medicine Diagnostic Reference Laboratory (TMDR) will be the central reception point for all blood samples collected from FTMCTU, and will follow a sample management system designed by the MORU Sample Management Centre.

The investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH Good Clinical Practice (ICH GCP) E6 (R2) and regulatory and institutional requirements for safety and protection of confidentiality of participants. The Co-Principal Investigators are responsible for data management and for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The study staff will enter the data into the participants' CRFs, which will be in a paper and/or electronic format. This includes safety data, laboratory data (both clinical and immunological) and outcome data. Data will be managed and stored in MACRO® database, a GCP-compliant electronic data capture system. A study data management plan will outline detailed procedures for data capture, storage, curation and preservation.

The documentation, data and all other information generated will be held in strict confidence. Only authorized, trained study staff as noted on the delegation log will have access to study records. The investigators will permit authorized representatives of the sponsor, ethical committee(s), regulatory authorities (if applicable), authorized representative of sponsor, 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. No information concerning the study or the data will be released to unauthorised third parties, without prior written approval of the Sponsor.

B6.5 Data analysis

A brief overview of the analytical plan is given below. A detailed Statistical Analysis Plan (SAP) will be developed as a separate document and finalized before database lock.

Proportions

Frequency data will be compared using chi squared or Fisher's exact test, as appropriate. Crude proportions will be calculated with the exact 95% confidence intervals (CI), where relevant.

Continuous data

For the primary efficacy outcome, the geometic means 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 MIST3 SAP. qPCR data points which, based upon the geometric mean of the three replicates, are negative or below the limit of quantification will be handled as specified in the MIST qPCR SAP. 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 the 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 the F-test. Test of significance will be performed at 5% significance level. Data will be analysed in Stata version 16 and above or using a comparable standard statistical analysis software. This will be detailed in the Statistical Analysis Plan.

B7. DATA AND SPECIMEN STORAGE AND/OR SHARING

B7.1 Management of specimen/data archiving and management of left-over samples

Specimen storage

With the participants' informed consent, any leftover cells and serum will be stored for 10 years in the FTM/MORU Sample Management System for future immunological and genetic analysis of malaria-specific responses.

Data handling and record keeping

Medical records and essential documents will be kept at the study site for approximately 5 years after study completion. Files containing identifiable information will be stored separate from other study data, in secure locations. Data recorded on paper records such as clinical worksheets and laboratory results printouts will be stored in locked cabinets. Electronic data will be stored on password controlled computers and in the study database that is hosted on a secure, access-controlled server in MORU. Physical access to the server is restricted to authorised and authenticated individuals only. A key card with special permissions is required to gain access to the server room . Only the Sponsor's representative, Investigators, the clinical

monitor, the ethical committee(s) and the regulatory authorities will have access to these records.

Data recorded in the CRF will be entered into the study database for storage and analysis. Personal identifying information such as names and telephone numbers will not be stored in the study database or used for analysis. Data will be pseudonymized, each participant will be identified by a unique study ID. Pseudonymised data will be stored indefinitely.

B7.2 Specimen/data sharing plan

With participants' consent, de-identified data and results from blood analyses stored in our database may be shared with other researchers to use in the future. Access to study data will be provided following the MORU data sharing policy.

With participants' consent, specimen/leftover specimen from this research project may be stored for 10 years for future use in the scope related or not related to this project.

If any data and/or specimen/leftover specimen from this current study to be used in any future research project, the investigator will submit the project to the Ethics Committee affiliated to the investigator for due consideration before use.

PART C: ETHICAL CONSIDERATION (DESCRIBE ONLY THE RESPONSIBILITIES OF THE PI)

C1. SIGNIFICANCE OF THE STUDY

This study is carried out to determine the safety, immunogenicity and efficacy of the PvDBPII/Matrix-M vaccine for *P. vivax* malaria.

C2. BALANCE OF RISK AND BENEFIT

C2.1 Risk of the study

This section describes the foreseen risks to the participant including risk related to:

Phlebotomy

There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venipuncture, which will not be documented as AEs if they occur.

PvDBPII/Matrix-M™ vaccination

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 participants 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 (24), as well as data available from more than 37,734 individuals who have previously received vaccines formulated in Matrix-M1, reversible reactogenicity will occur at a higher rate in subjects receiving the Matrix-M-adjuvanted vaccine than among those receiving vaccine with the same antigen without the adjuvant or placebo, although the reactogenicity is transient and generally mild or moderate in severity. The following adverse event profile is expected following vaccination with PvDBPII-Matrix-M1:

Local adverse events (AEs) such as injection site pain would be foreseeable to occur frequently. Less frequent local AEs 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 occur at higher rates among adjuvanted vaccinees, when compared to placebo vaccinees at an excess of ≥ 1% of subjects.

Inoculation

This controlled blood-stage challenge will involve a transfusion of a blood inoculum. As with any transfusion, there are risks of blood-borne infections and allergic reaction to blood products. The risk of blood-borne infection was reduced during the collection of blood inoculum in MIST 1 by extensive screening for blood-borne infection in all three of: the source patients; the CHMI infected blood donation volunteers from MIS1; and the stored inoculum. Serious allergic reactions including anaphylaxis have not been seen in challenge studies to date, but for safety reason participants will be inoculated in an area where physicians and a defibrillator are immediately available.

As the inoculum is prepared from packed red cells, the participants have a risk of developing a transfusion reaction. This risk has been minimized by preparing the inoculum from donors with blood Group O (universal donors), and the blood compatibility between the inoculum and volunteers will be checked. In addition, the volume of blood to be used (0.5 mL packed red cells) is much smaller than that given in transfusion of one unit packed red cells (470 mL).

Nevertheless, the participants will be monitored for this possibility during the first hour after the administration of the inoculum.

Plasmodium vivax infection

Base on previous studies, volunteers are likely to develop symptomatic malaria infection following challenge (25). The participants will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the study schedule. We will admit the participants to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University for close clinical monitoring, treatment (antimalarials) and symptomatic relief. A very small proportion of volunteers in previous *P. vivax* blood-stage and sporozoite-stage challenge studies have temporarily required intravenous fluid therapy for nausea and vomiting prior to treatment.

C2.2 Preventive and alleviative measures for risk

Phlebotomy

Participants' blood samples will be collected by well - trained medical personnel with aseptic technique to prevent infection or complications.

PvDBPII-Matrix-M™ vaccination

Participants will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any immediate postvaccination serious adverse reactions. Participants will be observed closely for at least 30 minutes following administration of the vaccine.

Plasmodium vivax infection

Participants will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the intensive follow-up schedule.

As described above, the participants will be hospitalized in the Hospital for Tropical Medicine, Faculty of Tropical Medicine, Mahidol University for close clinical monitoring, antimalarial treatment and symptomatic relief (e.g. fluids, analgesia and/or anti-emetics) until parasitaemia becomes negative. The treatment algorithm as well as the hospital guideline will be fully reviewed and strictly followed. The hospital will be officially informed prior to study initiation to ensure all the required medical facilities are in place. Clinical malaria expert at the Faculty of Tropical Medicine will be officially informed prior to study initiation. The medical monitors will be reachable throughout the whole study process.

In addition, this blood-stage inoculation will cause *P. vivax* infection without liver-stage parasites, thus removing the risk of relapse.

C2.3 Benefits of the study

There are no direct benefits to the participants taking part in this study. The expected benefit is to provide information about the safety and tolerability of the PvDBPII/Matrix-M™ vaccine compared to controls (placebo vaccine) in a *P. vivax* blood-stage controlled human malaria infection model and immunology data. All of these will benefit patients with malaria infections and population who are at risk of malaria infection in the future.

C3 SAFETY

Participant safety is of paramount importance, and we will follow these guidelines. The following measures are in place to safeguard participant safety:

- Participants will only be enrolled in the study if investigator judges this is appropriate.
- Participants' understanding of the trial information will be tested **by means of a questionnaire** at screening. This provides further confidence that fully informed consent has been obtained.
- Before challenge, full contact details for each participant will be documented, including home address and mobile telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the participants are easily contactable. Home and work landline telephone numbers where available and next-of-kin address and telephone numbers will also be documented. Participants must also provide the Investigators with the name and 24 hour telephone number of a close friend(s), relative(s) or housemate(s) who live nearby and who will be kept informed of their whereabouts for the duration of the study.
- The participants will be hospitalized when malaria parasite is identified (by qPCR) and will stay in the hospital until clinically recovered **AND** the completion of the antimalarial course (chloroquine) **AND** until two consecutive negative blood films.
- Participants will be able to contact a medically qualified member of the study team 24 hours a day throughout the study period.

Expectations of participant events following blood stage challenge

This part of the protocol aims to clearly review the events related to experience from the previous control human challenge studies in order to foresee the possible event(s) after the challenge that we may have to prepare to encounter within this study.

- According to references No. (25-28) and personal communication with other investigators, all volunteers in *P. vivax* blood stage challenge studies developed infection within 3 weeks

following the challenge. Most of AEs were mild to moderate grade and all were transient. No serious adverse events were reported.

C3.2 Safety Monitoring

C3.2.1 Medical Monitor

A Medical Monitor group, representing the Sponsor, will be appointed for oversight of safety in this clinical study. The Medical Monitor group will be responsible for safety assessments. The monitor will review the study prior to initiation and will be available to advise the Investigators on study-related medical issues and to act as a representative for the welfare of the subjects. The medical monitor does not have direct involvement in the conduct of the study. All serious adverse events will be reported to the medical monitor within 24 hours of becoming aware of the event. The medical monitor group is responsible for the review of the safety data and communicate with the PI and/or the DSMB, as appropriate.

The appointed medical monitor for the MIST 3 study is Dr. Lorenz Von Seidlein M.D.

C3.2.2 Data and Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) will review the study prior to initiation; review the interim safety data reports; and review all serious adverse events (SAEs) according to DSMB charter. The Board may convene additional reviews if deemed necessary, on review of the safety data, as sent, periodically by the medical monitor. All SAEs will be reported by the Co-Principal Investigator(s) to the DSMB at the same time as they are submitted to the ethics committees. The Co-Principal Investigator(s) will notify the Board and obtain a recommendation concerning continuation, modification, or termination of the study. The Co-Principal Investigator(s) will submit written DSMB summary reports with recommendations to the ethics committees(s). The roles and responsibilities of the DSMB will be formalised in a charter agreed with the members of the DSMB.

The DSMB will be independent of the Clinical Trials and Research Governance, Oxford University (Sponsor) and MIST programme team. The DSMB is charged with monitoring and evaluating the clinical data generated by this study, with a focus on safety, in and independent and objective manner. The main roles and responsibilities of the DSMB, DSMB procedures, and the DSMB meeting schedule are defined in the Data and Safety Monitoring Board Charter for the Malaria Infection Study in Thailand (MIST) Programme.

C4. CONSIDERATION FOR VULNERABLE RESEARCH PARTICIPANTS

Check whether your study involves any of the following vulnerable research participants.

☐ Prisoners

☐ Pregnant women

☐ Mentally ill persons

☐ Cancer or terminally ill patients

☐ Neonates/infants/children (aged <18)

☐ HIV/AIDS patients

☐ Institutionalized persons e.g. military, students, etc.

☐ Others (please specify)……..….

☑ Not applicable

C5. INFORMED CONSENT ISSUES

C5.1 Informed consent process

The informed consent process: The investigator will explain the purpose of the study with the help of and consistent with the Participant Information Sheet (PIS) prior to any study related procedures being undertaken. The participant must personally sign and date the latest approved version of the informed consent form (ICF) before any study specific procedures are performed.

The information sheet and informed consent form will be explained to the volunteers detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. The aims of the study and all procedures to be carried out will be explained. **It will be emphasised that there is no direct benefit from participating in the trial**.

- It will be clearly stated that participation is entirely voluntary and that refusing to participate will not involve any penalty or affect the participants' right to receive standard medical care.

- It will also be emphasized that if they do consent to participate and are enrolled, that they are free to withdraw from the study at any time, for any reason, without any penalty or prejudice to future care, and with no obligation to give the reason for withdrawal.

The volunteer will have the opportunity to question the investigator, or other independent parties to decide whether or not they will participate in the study.

If they do decide to participate, volunteers will be asked to **complete a questionnaire testing their understanding of the trial before signing the consent.** This helps to ensure that individuals understand the trial sufficiently to give informed consent. After the volunteer answers all questions in the questionnaire correctly, they will be asked to sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the investigator's site file and retained at the study site. These forms 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 following further discussion with the investigator. Only the subject who able to subsequently answer all questions in the questionnaire correctly will be asked to give the consent and will be screened for the trial. Only 2 attempts will be permitted in total.

Hospital consent for HIV test: This will be obtained prior to counselling and HIV test using the standard HIV consent from of Hospital for Tropical Diseases. Pre and post counselling for HIV screening and reporting will be conducted with the support of physician from the Hospital for Tropical Diseases. In case a volunteer is found to be HIV positive, follow up measures including but not limited to providing counselling and treatment according to standard hospital procedure will be arranged through a physician from the Hospital for Tropical Diseases.

C5.2 Informed consent documentation

Age	Informed Consent	Participant Information Sheet
20 years and over (Adult)	ICF for Adult Version 3.0 , dated 14 Dec 2022	PIS for Adult Version 3.0, dated 14 Dec 2022
	ICF for Data Sharing/ Data and Leftover Specimen Storage from Current Study for Future Use Version 3.0 , dated 14 Dec 2022	
	ICF for Leftover Specimen Storage from Current Study for future genetic analysis Version 3.0, dated 14 Dec 2022	
20 years and over (Adult)	HIV Pre-test counseling form, dated 1 October 2009	

C5.3 Compensation for research participants

☑ Yes, please provide details:

Participants will be compensated for their time and for the inconvenience caused by procedures as below (Table 8). This amount of compensation is calculated based on the cost of living in Bangkok for time and traveling. According to the consensus recommended by the controlled human challenge study group meeting in 2015, compensation specifically for risk was also advised (29).

Table 8*:* Estimated compensation amounts.

Activity	Compensation (THB)	Number of visits	Total (THB)
1. Screening visit (D-30)	2,000	1	2,000
2. 1stvaccination & $D_{3\&7\&14}$	1,500	4	6,000
3. 2ndvaccination & $D_{35\&42\&56}$	1,500	4	6,000
4. 3rdvaccination & $D_{217\&224}$	1,500	3	4,500

Activity	Compensation (THB)	Number of visits	Total (THB)
5. Admission per night (C_{-1}&C_0)	2,000	2 nights	4,000
6. Follow up (after challenge)	1,500	Around x 5 visits	7,500
7. Admission per night	2,000	Around x 10 nights	20,000
8. D_{Rx7}	1,500	1	1,500
9. C_{28}	1,500	1	1,500
10. C_{56}	1,500	1	1,500
11. C_{96}	1,500	1	1,500
12. C_{180}	1,500	1	1,500
13. C_{276}	1,500	1	1,500

Time in Trial (approx.)	Maximum no. of visits	Maximum volume of blood taken[1] (mL)	Total compensation amount (THB)
1 year	13	613 mL	59,000

Remark:

[1]Maximum volume of blood taken is 613 mL in case participant received antimalarial treatment on day 16.

In case a participant has to come for extra visit(s), they will be compensated for their time and for the inconvenience with 1,000 THB per day.

☐ No, please provide reasons: ……..……

C5.4 Responsible and contact persons

☑ Person(s) responsible for payment for treatment of complications and adverse effects

☑ Person(s) including doctor(s) and/or contact address(es) and telephone number(s) for emergency use

1. **Dr. Kittiyod Poovorawan**, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Tel. (02) 354-9100 ext. 1435, Mobile (083) 149-6864
2. **Dr. Podjanee Jittamala,** Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Tel. (02) 306-9157, Mobile (081) 956-3371.
3. **Dr. Borimas Hanboonkunupakarn**, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Tel. (02) 354-9100 ext. 3160, Mobile (086) 970-5705

PART D: APPENDIX A

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APPENDIX B: SUBMISSION PACKAGE INCLUDES

No.	Submitted Documents
1.	Research Proposal Submission Form for a study involving human subject enrollment WITH specimen collection (FTM ECF-033-RR)
2.	Informed Consent Form and Participant Information Sheet
3.	Informed Consent Form for Data Sharing/ Data and Leftover Specimen Storage from Current Study for Future Use
4.	Informed Consent Form for Leftover Specimen Storage from Current Study for future genetic analysis
5.	HIV Pre-test Counseling Form
6.	Questionnaire to evaluate participant's understanding of the study
7.	Recruitment materials
8.	Diary card
9.	Screening Form
10.	Case Record Form

APPENDIX C: SAFETY TERMS AND ADMINISTRATIVE INFORMATION

1. SAFETY DETECTION, ASSESSMENT, DOCUMENTATION AND REPORTING

The investigators and designated site staff is/are responsible for the detection, assessment, documentation and reporting of events meeting the criteria and definition of an adverse event (AE), or serious adverse event (SAE) as provided in this protocol. Participants will be instructed to contact the investigator immediately should the participant manifest any signs or symptoms they perceive.

1.1 Definitions

1.1.1 Adverse Event (AE):

An AE is any untoward medical occurrences in a participant, which may occur during or after administration of a study intervention (in this case, vivax parasite challenge) and does not necessarily, have a causal relationship with the intervention. An AE can therefore be any unfavorable 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. Any events occurring between screening and C_{-1} will be considered as baseline/, pre-existing conditions. This information will be recorded in the medical records.

1.1.2 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 (i.e. results in death from any cause at any time).
- Life-threatening event (i.e. the participant 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 serious 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).
- Transfer of inpatient care to the intensive care unit, if the Investigators assess that a higher level of intervention and intense monitoring is required to manage symptoms following controlled human malaria infection, or drugs (over and above what can be provided by the Investigators in research bay). Hospitalization (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a SAE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the

participant and/or require medical or surgical intervention to prevent one of the outcomes listed above.

- Congenital anomaly or birth defect.

All pregnancies occurring in participants within 3 months of challenge will be considered a SAE.

1.2 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events:

In absence of a diagnosis, clinically abnormal laboratory findings (e.g. clinical chemistry, haematology, and urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as an AE or SAE if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

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 participant will be informed and appropriate medical care arranged as appropriate with the permission of the participant. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Investigator.

The study will cover all expense of the treatment for SAE.

1.2.1 Causality assessment

The investigator is obligated to assess the relationship between study procedure and/or antimalarial medications and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study procedure and antimalarial medications will be considered and investigated. The relationship of the adverse event with the study procedures will be categorized as unrelated, unlikely to be related, possibly related, probably related or definitely related (Table C1). An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The delegated clinician will use clinical judgment to determine the relationship.

Table C1: Guidelines for assessing the relationship of study intervention to an AE

0	Unrelated	No temporal relationship to study intervention and Alternate aetiology (clinical state, environmental or other interventions); and Does not follow known pattern of response to CHMI, blood donation or drug.
1	Unlikely to be related	Unlikely temporal relationship to study intervention and Alternate aetiology likely (clinical state, environmental or other interventions); and Does not follow known typical or plausible pattern of response to challenge, blood donation or drug.
2	Possibly related	Reasonable temporal relationship to study intervention; or Event not readily produced by clinical state, environmental or other interventions; or Similar pattern of response to that seen with previous challenge, blood donation or similar drug.
3	Probably related	Reasonable temporal relationship to study intervention; and Event not readily produced by clinical state, environment, or other interventions; or Known pattern of response seen with previous challenge, blood donation or drug.
4	Definitely related	Reasonable temporal relationship to study intervention; and Event not readily produced by clinical state, environment, or other interventions; and Known pattern of response seen with previous challenge, blood donation or drug.

1.2.2 Assessment of AE intensity

Each adverse event will be graded by the investigator and designated study staff according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

In the rare case that an adverse event is not graded in the CTCAE, then that event should be graded as follows:

Grade 1: Mild AE

Grade 2: Moderate AE

Grade 3: Severe AE

Grade 4: Life-threatening or disabling AE

Grade 5: Death related to AE

All AE/SAE will be collected throughout the first 3 month after challenge or until a satisfactory resolution occurs. All AEs that result in a participant's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the participant consents to this). In cases where the participant has to do AE self-detection and self- assessment, a simple grading system for the self-assessment on the diary card will be used (Table C2).

Table C2: Severity grading criteria for the self-assessment for AEs.

GRADE 0	None
GRADE 1	Noticeable, but no impact on activities
GRADE 2	Noticeable and alters, but does not prevent daily activities
GRADE 3	Prevents normal daily activities, and/or requires visits with a medical professional
GRADE 4	Precipitates ER visit or hospitalization (these are, of course, also SAEs; Serious Adverse Effects).

1.2.3 Assessment of outcomes

The investigator will assess the outcome of all AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

During the inpatient phase, the adverse events (including all SAEs) will be assessed by the investigator and the delegated study staff.

While the participant is followed as an outpatient, a diary card will be provided, and the participant will be instructed how to self-assess the cause and severity of AEs. This self-assessment symptom diary will be checked, discussed, collected and recorded at each clinic visit.

For all AEs , appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilized, or a non-trial related causality has been assigned.

1.3 Reporting procedures

For SAEs

In order to comply with current regulations on serious adverse event reporting to Ethics and regulatory authorities (if applicable), the event will be documented accurately and notification deadlines respected.

SAEs will be reported to the medical monitor immediately (within 24 hours) of the Investigators' being aware of their occurrence.

SAEs will also be reported to ethics committees, the Data and Safety Monitoring Board (DSMB), and the regulatory authority (if applicable), in accordance with reporting requirements and according to required timelines.

1.4 Events or outcomes not qualifying as adverse events or serious adverse events

Pregnancy

Participants are informed that for the safety of participant and their child, pregnancy and breastfeeding are prohibited until 3 months after the challenge. Female study participants are asked to use appropriate contraceptive methods to prevent pregnancy while they participate in the studyfrom the first vaccination until 3 months after the injection of malaria infected erythrocytes.

Contraceptive methods include:

- Established use of oral, injected or implanted hormonal contraceptives
- Intrauterine Device or Intrauterine System
- Barrier methods (condoms or diaphragm with additional spermicide)
- Male sterilisation and female sterilisation (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate)

- True abstinence, when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Female participants will be tested for pregnancy at screening, prior to receive each vaccine dose, prior to challenge, prior to receive antimalarial drug, and during follow up as indicated in the schedule of procedures (Tables 4 and 5).

Should a participant become pregnant during vaccination she will be winthdrawn from the study, if she becomes pregnant after challenge, she will be treated with antimalarials immediately and will be withdrawn from the study. We will not routinely perform venepuncture on such participants, other than blood films to check that any parasitaemia has been cleared by the antimalarial treatment. With the participant's permission she shall be followed up until pregnancy outcome. The management of any participant 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 consultants at Faculty of Tropical Medicine and the Mahidol Oxford Tropical Medicine Research Unit, including advice on antimalarial drug choice.

Should a participant become pregnant after receiving antimalarial treatment (but prior to the end of the study), they shall be discontinued from the study as soon as we have confirmed that their parasitaemia has cleared. The participant will still be followed up until 3 months after challenge and pregnancy outcome will be followed up. For male participant whose partner become pregnant during the study, the partner will be followed up pregnancy outcome.

During pregnancy the following should always be considered as an SAE:

- Spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
- Ectopic and molar pregnancy
- Stillbirth (intrauterine death of fetus after 22 weeks of gestation).
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect identified in the offspring of a study participant (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

However, all pregnancies occurring in participants within 3 months of challenge will be considered a SAE.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the study procedures will be reported. While the

investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

Male study participants are asked to use condom during sexual intercourse or abstinent to prevent pregnancy in their partners while they participate in the study from the first vaccination until 3 months after the injection of malaria infected erythrocytes.

All pregnancies occurring during the study will be reported to the DSMB.

1.5 Safety monitoring

Monitoring

Monitoring will be performed using an established Monitoring Plan. Independent monitoring will be performed by Clinical Trials Supporting Group (CTSG), MORU. 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 Good Clinical Practice (GCP). The investigators will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the Sponsor and inspection by local and regulatory authorities.

2. RANDOMIZATION

This will be a randomized double-blinded controlled design. Volunteers who fulfil the inclusion criteria and have none of the exclusion criteria will be randomised in a 1:1 ratio to either the study vaccine group or the sham vaccine control group in accordance with the randomisation schedule. Randomisation will be in blocks of 8. Allocation will be done by drawing the next sequential numbered opaque envelope, which contains the study number and treatment allocation. The randomization list and numbered envelopes will be prepared by members of the MORU CTSG statistical staff.

Only the CTSG statistical staff and the study pharmacist will know the study group allocation of each participant. The study pharmacist will prepare the syringes containing either the vaccine or 0.9% saline. These individuals will not be involved in providing participant care, conducting study investigations, or performing laboratory tests necessary for determining the study endpoint. They will not be involved in any other study assessment or evaluation.

3. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures and prespecified monitoring plan, the monitors will verify that the clinical study is conducted and data are generated, documented and

reported in compliance with the current approved protocol, standard operating procedures, ICH GCP and relevant regulations.

The Sponsor and authorised individuals may carry out audit to ensure compliance with the protocol, standard operating procedures, ICH GCP and relevant regulations. The study audits will be managed by an independent function according to standard operating procedures a prespecified audit plan.

GCP inspections may also be undertaken by the regulatory authority or ethical committee(s) to ensure compliance with protocol and national regulations. The sponsor will assist in any inspections.

4. ETHICS

Ethical approval will be sought prior to commencing the study through the relevant Research Ethics Committees. Indemnity for the trial will be provided by the University of Oxford. SAEs will be reported to the medical monitor, DSMB, and the ethics committees. GCP certificate will be obtained by all staff/investigators prior to commencing the studies.

The Investigators will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki 2013. The trial will adhere to the ICH GCP E6 (R2).

The protocol, informed consent form, participant information sheet, and other written participant information/materials and Advertisement will be submitted to appropriate Research Ethics Committees (RECs), and regulatory authorities (if applicable) for written approval. The Co-Principal Investigator (Co-PI) will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents. The Investigator will notify deviations from the protocol or SAEs occurring at the site to REC(s) in accordance with procedures.

The Co-Principal Investigator shall submit a report once a year throughout the study, or on request, to the ethic committees. In addition, an End of Study notification and final report will be submitted to the ethic committees.

5. SPONSOR: UNIVERSITY OF OXFORD

Contact information: University Offices, Wellington Square, Oxford, OX1 2JD, United Kingdom

The study sponsor has a role in the design of the study; collection of data; management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

6. FUNDER

The funder, the Welcome Trust, had no role in the design of the study. The funder will not have any role in its execution, analyses, and interpretation of data or decision to submit results.

7. TRIAL COMMITTEES

Programme Steering Committee (PSC): The role of PSC is to provide overall supervision for the project on behalf of the Project Sponsor and Project Funder and to ensure that the project is conducted to the rigorous standards set out in the Research Governance Framework for Health and Social Care, and in the Guidelines for Good Clinical Practice. The composition and roles and responsibilities of the PSC, and the PSC meeting schedule, are defined in the PSC Terms of Reference.

8. INSURANCE

The University of Oxford has a specialist insurance policy in place - Newline Underwriting Management Ltd, at Lloyd's of London – which would operate in the event of any participant suffering harm as a result of their involvement in the research.

9. PUBLICATION POLICY

All Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged.

The trial results for each participant will be communicated individually to that individual to inform his/her health history.

10. IMMUNOLOGICAL ASSAYS (BLOOD VOLUME TABLE)

10.1 Immunological assays (humoral & cellular responses)

Time point	Sample	Amount	Test
Vaccination D0	Serum	10ml	Binding inhibitory titers, Antibody titers, IgG purification for growth inhibition assays (control for all assays – therefore large vol required)
	Plasma	2ml	Cytokines/chemokine by Luminex (control for all assays)
	WB Tempus	2ml	Gene expression by NanoString (control)
	WB Cyto	2ml	Immune cell populations by flow cytometry (control)
Vaccination D3	Plasma	2ml	Cytokines/chemokine by Luminex
	WB Tempus	2ml	Gene expression by NanoString

Time point	Sample	Amount	Test
	WB Cyto	2ml	Immune cell populations by flow cytometry
Vaccination D7	Plasma	2ml	Cytokines/chemokine by Luminex
	WB Tempus	2ml	Gene expression by NanoString
	WB Cyto	2ml	Immune cell populations by flow cytometry
Vaccination D14	Serum	2ml	Antibody titers
Vaccination D35	Plasma	2ml	Cytokines/chemokine by Luminex
	WB Tempus	2ml	Gene expression by NanoString
	WB Cyto	2ml	Immune cell populations by flow cytometry
Vaccination D42	Serum	5ml	Antibody titers, Antibody affinity
Vaccination D210	Serum	2ml	Antibody titers, Antibody affinity
	Plasma	2ml	Cytokines/chemokine by Luminex
	WB Tempus	2ml	Gene expression by NanoString
	WB Cyto	2ml	Immune cell populations by flow cytometry
Vaccination D217	Plasma	2ml	Cytokines/chemokine by Luminex
	WB Tempus	2ml	Gene expression by NanoString
	WB Cyto	2ml	Immune cell populations by flow cytometry
C-1	Serum	10ml	Binding inhibitory titers, Antibody titers, isotyping, avidity, antibody affinity, C1q and Fc receptor binding (including FcγRI, FcγRIIA, FcγRIIB, FcγRIIIA, FcγRIIIB, Fcα and μ), cellular phagocytosis (antibody dependent cellular phagocytosis, ADCP), neutrophil burst (antibody dependent neutrophil burst, ADNB), complement deposition (ADCD) and NK cell activation (ADNKA)
	WB for PBMCs for mAbs	30ml	Monoclonal antibody development
C+14	Serum	2ml	Antibody titers
C+21	Plasma	2ml	Cytokines/chemokine by Luminex
	WB Tempus	2ml	Gene expression by NanoString
	WB Cyto	2ml	Immune cell populations by flow cytometry
C+28	Serum	2ml	Antibody titers
C+56	Serum	2ml	Binding inhibitory titers, Antibody titers
C+96	Serum	2ml	Binding inhibitory titers, Antibody titers

10.2 Immunological functional assays (ELISA&GIA)

Time point	Sample	Amount	Test
Vaccination D0	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA

Time point	Sample	Amount	Test
	Clot blood	6ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D14	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D28	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D35	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D42	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D56	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D210	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D217	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
Vaccination D224	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
	Clot blood	6ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
C-1	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
	Clot blood	6ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
C7	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
C14	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
D reach treatment	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
C56	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
C96	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA
C276	Clot blood	4ml	Humoral and B cell responses to the *P. vivax* Duffy-binding protein region II (PvDBPII) by ELISA

10.3 Immune cells & cytokines profile (phenotypic profile)

Time point	Sample	Amount	Test
D0	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D7	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D14	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D28	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D35	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D42	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D210	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D217	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
D224	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
C-1	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile
C21	EDTA blood	2ml	Host response to the blood stage infection by phenotypic profile of the immune cells and cytokines profile

APPENDIX D: LIST OF ABBREVIATIONS

AE	adverse event
β-HCG	beta–Human Chorionic Gonadotropin
BP	blood pressure
BT	body temperature
BUN	blood urea nitrogen
CBC	complete blood count
CBF	Clinical Bio-Manufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CHIK	Chikungunya
CHMI	controlled human malaria infection
CI	Chief Investigator
CMV	Cytomegalovirus
Cr	Creatinine
CRF	case record form
CSP	Circumsporozoite
CTCAE	Common Terminology Criteria for Adverse Events
CTSG	Clinical Trials Supporting Group
CYP2D6	enzyme predicting Primaquine metabolism (Cytochrome p450 2D6)
DARC	Duffy antigen receptor for chemokines
DBP	Duffy binding protein
DEN	Dengue

DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
ELISA	Enzyme-linked Immunosorbent Assay
ECG	Electrocardiogram
ELISPOT	Enzyme linked immunospot assay
EPI	Expanded Program of Immunization
FBS	fasting blood sugar
FDA	United States Food and Drug Administration
FTM	Faculty of Tropical Medicine
FTMCTU	Clinical Therapeutics Unit (healthy volunteer ward), Faculty of Tropical Medicine
FTMEC	Ethics Committee, Faculty of Tropical Medicine
G6PD	glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HLA	Human Leukocyte antigen
HTLV	Human T cell Lymphotrophic virus
ICF	Informed Consent Form

ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IFN-α	Interferon-gramma
IM	Intramuscular
IMP	Investigational Medicinal Product
JE	Japanese Encephalitis
KEMRI	Kenya Medical Research Institute
LFT	liver function test
LPS	Lipopolysaccharide
MFA	Membrane Feeding Assay
MORU	Mahidol Oxford Tropical Medicine Research Unit
MVRU	Mahidol Vivax Research Unit
MSP	Merozoite surface proteins
µg	Microgram
OxTREC	Oxford Tropical Research Ethics Committee
PCR	polymerase chain reaction
P. falciparum	Plasmodium falciparum
PI	Principal Investigator
PIS	Participant Information Sheet
PMR	parasite multiplication rate
PQ	Primaquine
PR	pulse rate

P. vivax	Plasmodium vivax
PvAMA1	*P. vivax* apical merozoite antigen 1
PvCSP	*P. vivax* circumsporozoite
PvDBPII	receptor-binding region II of *P. vivax* Duffy binding protein
PvRBP	*P. vivax* reticulocyte binding protein
PvTRAP	*P. vivax* thrombospondin related adhesive protein
QIMR	Queensland Institute of Medical Research
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
REC	Research Ethics Committee
RR	respiratory rate
RUNMC	Radboud University Nijmegen Medical Centre
SAE	serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
TMDR	Tropical Medicine Diagnostic Reference Laboratory
USMMVP	U.S. Military Malaria Vaccine Program
VLP	Virus-like particle
WHO	World Health Organization
WI	Work Instruction
ZIK	Zika virus

APPENDIX E: AMENDMENT HISTORY

Amendment No.	Proposal Version No.	Date issued	Author(s) of changes	Details of Changes made
NA	NA	NA	NA	NA