A Phase Ia study to assess safety and immunogenicity of the *Plasmodium falciparum* malaria vaccine candidate Pfs48/45 in Matrix-M adjuvant in healthy adults living in the UK

Study Reference: VAC085

Protocol Version: 2.0

Date: 11 January 2023

IRAS Project ID: 1004817

REC reference: 22/SC/0291

EudraCT ID: 2021-006763-26

NCT05400746

Chief Investigator: Angela M Minassian

Sponsor: University of Oxford

MODIFICATION HISTORY

Version	Date	Author(s)	Modifications
1.0	22 March 2022	Cyndi Goh David Mekhaiel Angela Minassian Sumi Biswas	Created
1.1	11 May 2002	Cyndi Goh	Wording of group holding rules amended as per MHRA request
2.0	11 January 2023	Angela Minassian	Rewording of 14.5 Storage and use of data Rewording of 8.2.4 Vaccination and re-vaccination exclusion criteria Rewording of 9.2.5 Management of post-vaccination fever wording Bleed table pg 45 – D70 plasma and serum tube numbers. Total blood draw unchanged. Update to sponsor email address Update PHE to UK HSA

A Phase Ia study to assess safety and immunogenicity of the *Plasmodium falciparum* malaria vaccine candidate Pfs48/45 in Matrix-M adjuvant in healthy adults living in the UK

Study Code: VAC085

Chief Investigator	Dr Angela M Minassian Centre for Clinical Vaccinology and Tropical Medicine, Jenner Institute, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE Email: angela.minassian@ndm.ox.ac.uk
Investigator	Prof Sumi Biswas Jenner Institute, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Headington, Oxford, OX3 7DQ Email: sumi.biswas@ndm.ox.ac.uk
Trial Site	Centre for Clinical Vaccinology & Tropical Medicine Jenner Institute, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE PI at Site: Dr Angela M Minassian
Sponsoring Institution	University of Oxford Research Governance, Ethics and Assurance Team, Research Services, University of Oxford Joint Research Office, Boundary Brook House Churchill Drive, Headington, Oxford, OX3 7GB Tel: 01865 616480 Email: rgea.sponsor@admin.ox.ac.uk

External Monitor	Appledown Clinical Research Ltd. Orchard End, Greenlands Lane, Prestwood, Bucks, HP16 9QX Tel: 01494 867676/07941 271635 Email: ceri.mckenna@appledown.net
Local Safety Monitor	Prof Brian Angus Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE Tel: 01865 220289 Email: brian.angus@ndm.ox.ac.uk
Funder	European Commission (Horizon 2020) Rue de la Loi 200 / Wetstraat 200 1040 PO Box 1049 Brussels Belgium The University of Oxford Internal Funds Nuffield Department of Medicine (NDM) Henry Wellcome Building for Molecular Physiology Old Road Campus, Headington Oxford, OX3 7BN

Confidentiality Statement

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

Investigator Agreement

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

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

Dr Angela Minassian	[redacted for public release]	13 Jan 2023
Chief Investigator	Investigator Signature	Date
Dr Angela M Minassian		

Conflict of Interest

1. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no/the following (delete as appropriate) conflict of interest"

Dr Angela Minassian	[redacted for public release]	
		13 Jan 2023
Chief Investigator	Investigator Signature	Date
Dr Angela M Minassian		

Details: n/a

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

	[redacted for public release]	
Prof Sumi Biswas		13 Jan 2023
Investigator	Investigator Signature	Date
Prof Sumi Biswas		

Details: n/a

Table of Contents

1 SYNOPSIS

Title	A Phase Ia study to assess safety and immunogenicity of the *Plasmodium falciparum* malaria vaccine candidate Pfs48/45 in Matrix-M adjuvant in healthy adults living in the UK
Trial Centre	Clinical Centre for Vaccinology and Tropical Medicine, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE, UK
Trial Identifier	VAC085
Clinical Phase	Phase Ia
Design	Open label, single-site, first-in-human, dose-escalation Phase Ia study
Population	Healthy adults aged 18 – 45 years
Sample Size	Total: 24-30 volunteers Group 1: 8-10 volunteers receiving three doses of 10 µg Pfs48/45 in 50 µg Matrix-M on days 0, 28 and 56 Group 2: 8-10 volunteers receiving three doses of 50 µg Pfs48/45 in 50 µg Matrix-M on days 0, 28 and 56 Group 3: 8-10 volunteers receiving two doses of 50 µg Pfs48/45 in 50 µg Matrix-M on days 0 and 28, followed by one dose of 10 µg Pfs48/45 in 50 µg Matrix-M on day 56
Follow-up duration	Participants will be followed for approximately 8 months
Planned trial period	Approximately 12 months from first vaccination of the first volunteer
Primary Objective	To assess safety and tolerability of the Pfs48/45 in Matrix-M vaccine in healthy adult volunteers
Secondary Objectives	To assess the humoral and cellular immunogenicity of the Pfs48/45 in Matrix-M vaccine, when administered to healthy adult volunteers in the various regimes To assess *ex vivo* efficacy of the Pfs48/45 in Matrix-M vaccine using membrane feeding assays, when administered to healthy adult volunteers

Investigational Products	1. Pfs48/45 – recombinant protein transmission-blocking malaria vaccine candidate 2. Matrix-M – saponin based vaccine adjuvant
Form	1. Pfs48/45: liquid, stored at -80 °C (nominal) 2. Matrix-M: liquid, stored at +2°C to +8°C
Dose	Group 1: a. Pfs48/45 - 10 µg, administered in a three-dose schedule on days 0, 28 and 56 b. Matrix-M – 50 µg, administered with each Pfs48/45 dose on days 0, 28 and 56 Group 2: a. Pfs48/45 – 50 µg, administered in a three-dose schedule on days 0, 28 and 56 b. Matrix-M – 50 µg, administered with each Pfs48/45 dose on days 0, 28 and 56 Group 3: a. Pfs48/45 – 50 µg, administered in a two-dose schedule on days 0 and 28 followed by 10 µg administered in a one-dose schedule on day 56 b. Matrix-M – 50 µg, administered with each Pfs48/45 dose on days 0, 28 and 56
Route	Intramuscular injection (IM) in the deltoid region of the arm

2 ABBREVIATIONS

ACT	Artemisinin-combination therapies
AE	Adverse event
ALS	Advanced life support
AR	Adverse reaction
ß-hCG	Beta human chorionic gonadotropin
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CD1	Cluster of differentiation 1
ChAd63	Chimpanzee adenovirus 63
CI	Chief investigator
COVID-19	Coronavirus disease 2019
CRF	Case report form
DMFA	Direct membrane feeding assay
DNA	Deoxyribonucleic acid
DSUR	Development safety update report
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot assay
EM	Electron microscopy
FDA	United States Food and Drug Administration
GCP	Good clinical practice
GLURP-R0	Glutamate rich protein R0
GMP	Good manufacturing practice
GP	General practitioner
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G
IM	Intramuscular
IMP	Investigational medicinal product
kDa	Kilodaltons
LSM	Local safety monitor
mAb	Monoclonal antibody
MFA	Membrane feeding assay

MHRA	Medicines and Healthcare products Regulatory Agency
MPC	Multi-protein complexes
MVA	Modified vaccinia Ankara
NDM	Nuffield department of medicine
NK	Natural killer
P. falciparum	*Plasmodium falciparum*
P. vivax	*Plasmodium viax*
PCR	Polymerase chain reaction
PHE	Public Health England
PIS	Participant information sheet
PPE	Personal protective equipment
PPM	Parasite plasma membrane
PV	Parasitophorous vacuole
PVM	Parasitophorous vacuole membrane
QP	Qualified person
RBM	Roll back malaria
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance Team
RNA	Ribonucleic acid
S2	Schneider 2
SAE	Serious adverse event
SAR	Serious adverse reaction
SDEA	Safety Data Exchange Agreement
SMFA	Standard membrane feeding assay
SOP	Standard operating procedure
STOP-TRANS	Safety and efficacy of R0.6C vacccine
SUSAR	Suspected unexpected serious adverse reaction
TBI	Transmission blocking immunity
TBV	Transmission blocking vaccine
TOPS	The overvolunteering prevention system
TMF	Trial master file
TRA	Transmission reducing activity
UOXF	University of Oxford
VIMT	Vaccines to interrupt malaria transmission
VLP	Virus-like particle

WHO	World Health Organisation

3 BACKGROUND & RATIONALE

3.1 The need for a vaccine against *Plasmodium falciparum* malaria

Malaria is a potentially life-threatening parasitic infection and a major public health concern. Globally, 3.4 billion people are thought to be at risk of infection [1, 2], and in 2019 a reported 229 million cases and 409 thousand deaths occurred from this disease [3]. Children under five years of age are the most severely affected, with 67% of worldwide malaria-related deaths occurring in this age group in 2019 [3].

Of the five main species known to infect humans, *Plasmodium falciparum (P. falciparum)* is the most prevalent and the most important cause of morbidity and mortality. *Plasmodium vivax* also adds significantly to worldwide morbidity, with *Plasmodium ovale, Plasmodium malariae* and the zoonotic species *Plasmodium knowlesi* contributing to a lesser extent [4]. Geographically, *P. falciparum* is the predominant species in four of the five malaria endemic continents, and is overwhelmingly the primary malaria parasite in the WHO African Region, where it accounted for 99.7% of all malaria cases in 2018; the same region accounted for 94% of all malaria cases and deaths worldwide [5].

Since 2000, following concerted worldwide efforts, there have been huge gains in the global fight against all-cause malaria, leading to a reduction in overall mortality from 736,000 in 2000 to 409,000 in 2019 [3]. In the WHO African Region, malaria control interventions accounted for 70% of the 943 million fewer malaria cases occurring between 2001 and 2015, averting an estimated 663 million malaria cases [6, 7]. This has largely been achieved through increased coverage of insecticide treated nets, estimated to account for 68% of the averted cases, as well as increased accessibility to artemisinin-combination therapies (ACTs) and increased indoor residual spraying, estimated to have contributed 19% and 13% respectively to prevented cases [8]. Other interventions, such as increased use of rapid diagnostic tests and intermittent prophylactic therapy for pregnant women and seasonal prophylaxis for children also play an important role [9-15]. In addition, in low-transmission areas and regions where artemisinin-resistant strains are emerging, single, low-dose primaquine is now recommended, in combination with ACT, as a gametocytocidal agent for non-pregnant adults and children above one year of age with parasitological evidence of *P. falciparum* infection [16]. However, limited direct evidence for safety for use in populations with glucose-6-phosphate dehydrogenase deficiency (G6PDD) and lack of access to diagnostics for pre-treatment identification of G6PDD has resulted in ongoing safety concerns and poor levels of implementation. The high rates of gametocyte carriage in asymptomatic individuals in high-transmission settings are also considered too large for adjunctive primaquine therapy to have any impact, limiting its use for malaria control across many African settings [17].

Despite nearly 20 years of advances, the WHO reports that the rate of progress in both cases and deaths has slowed worldwide since 2014 [18]. In some cases, including the ten highest burden African countries, the rate of progress has reversed [19]. Furthermore, current strategies are complicated by the rise in insecticide-resistant *Anopheles* mosquitoes and antimalarial resistant parasite strains in South East Asia and China, underlining the need for new tools for malaria control [20].

The Roll Back Malaria (RBM) Partnership was launched in 1998 by the WHO, the United Nations Children's Fund, the United Nations Development Programme and the World Bank. A major goal of The RBM Partnership is to support the development of vaccines against malaria as a key future strategy for reducing mortality from malaria. The WHO Global Vaccine Research Forum has set out a strategic framework for malaria vaccine development in the Malaria Vaccine Technology Roadmap, defining the goals for global malaria vaccine development community [21]. The

Roadmap calls for the development of vaccines against both *P. falciparum* and *P. vivax* by 2030 to achieve two key objectives: protective efficacy of at least 75 percent against clinical malaria and reduction of parasite transmission to substantially reduce incidence of human malaria infection, enabling elimination in multiple settings.

3.2 Lifecycle of the *P. falciparum* malaria parasite

In the pre-erythrocytic stage of human infection, the bite of an infected female *Anopheles* mosquito transmits malaria sporozoites to the human host where they travel via the bloodstream to the liver and invade hepatocytes (liver-stage). Here, they mature into merozoites for 6 to 7 days, after which the hepatocytes rupture releasing thousands of merozoites into the bloodstream. Merozoites then invade erythrocytes where they multiply and after 2 days cause the erythrocyte to rupture, releasing progeny merozoites that in turn invade new erythrocytes (blood-stage). It is the blood-stage of infection that is associated with clinical disease and potentially severe or fatal complications. The liver stage is asymptomatic.

During the blood-stage infection, a small percentage of parasites undergo an alternative development pathway, differentiating into sexual male and female gametocyte forms. Over 5-12 days, the intra-erythrocytic gametocytes progress through four developmental forms (stages I–IV) before reaching maturity, as stage V gametocytes [22-24]. The earlier stages of sexual development are largely absent from peripheral blood and are thought to sequester extravascularly in the bone marrow, before returning to the circulation as mature Stage V gametocytes [25]. The mature gametocytes are infectious to the mosquito vector, permitting transmission from human to *Anopheline* host.

Following ingestion by the mosquito at the blood meal, environmental shifts, including changes in temperature and pH, as well as exposure to xanthurenic acid, trigger gametogenesis [26]. The female intra-erythrocytic sexual forms emerge as macrogametes, whilst male gametocytes undergo three rounds of DNA replication before exflagellation to release eight motile microgametes [24]. Extracellular macro and microgametes will unite within the mosquito's midgut to undergo fertilisation, forming zygotes, which go on to become motile ookinetes that traverse the midgut epithelium. At the basal lamina, following meiotic division, the ookinete further develops into the oocyst. Multiple further rounds of division follow, eventually culminating in rupture of the oocyst and release of sporozoites. The sporozoites subsequently migrate to the mosquito's salivary glands, where they will be injected into the human host at the next blood meal [22-24]. This lifecycle is summarised in Figure 1.

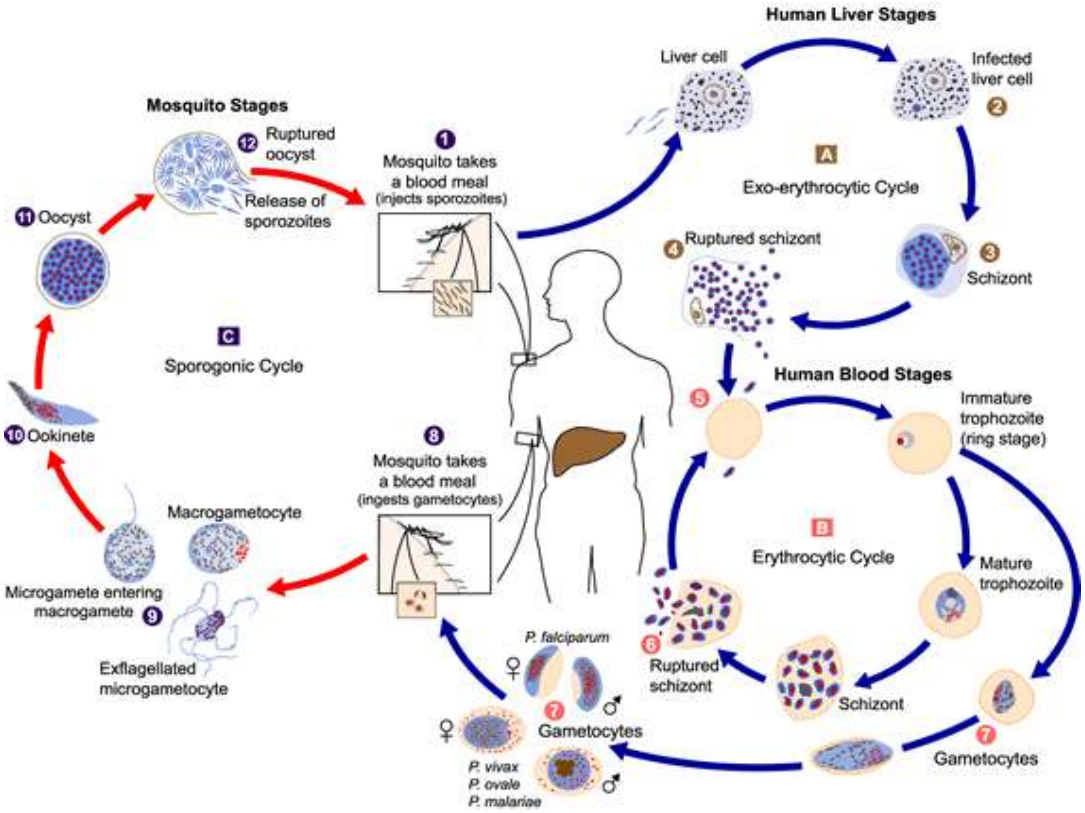

Figure 1: Lifecycle of *P. falciparum* malaria. Figure reproduced from the Centers for Disease Control and Prevention website [27].

3.3 Progress in vaccines targeting malaria

Malaria vaccines can be subdivided into three categories based on the stages of the parasite life cycle targeted: pre-erythrocytic (liver-stage) vaccines, blood-stage vaccines and transmission-blocking vaccines (Figure 2). Of the three stages, there has been most progress made with pre-erythrocytic vaccines; the malaria vaccine RTS,S/ASO1E [28, 29] received approval from the European Medicines Agency in 2015 and pilot implementation programmes amongst African children were launched by the WHO in 2019.

Since 2000, the rate of new malaria vaccine trials registered at ClinicalTrials.gov has remained steady at approximately 10 trials per year [30]. Initially, there was a strong focus on the subunit vaccine RTS,S. Over time, trials investigating whole-sporozoite pre-erythrocytic stage vaccines and transmission-blocking vaccines have increased in frequency.

There has been increasing interest in the use of vaccines for malaria elimination. A proposed vaccine to interrupt malaria transmission (VIMT) could include antigens expressed during the three stages of the parasite lifecycle in order to reduce or halt the spread of parasites in the community [31].

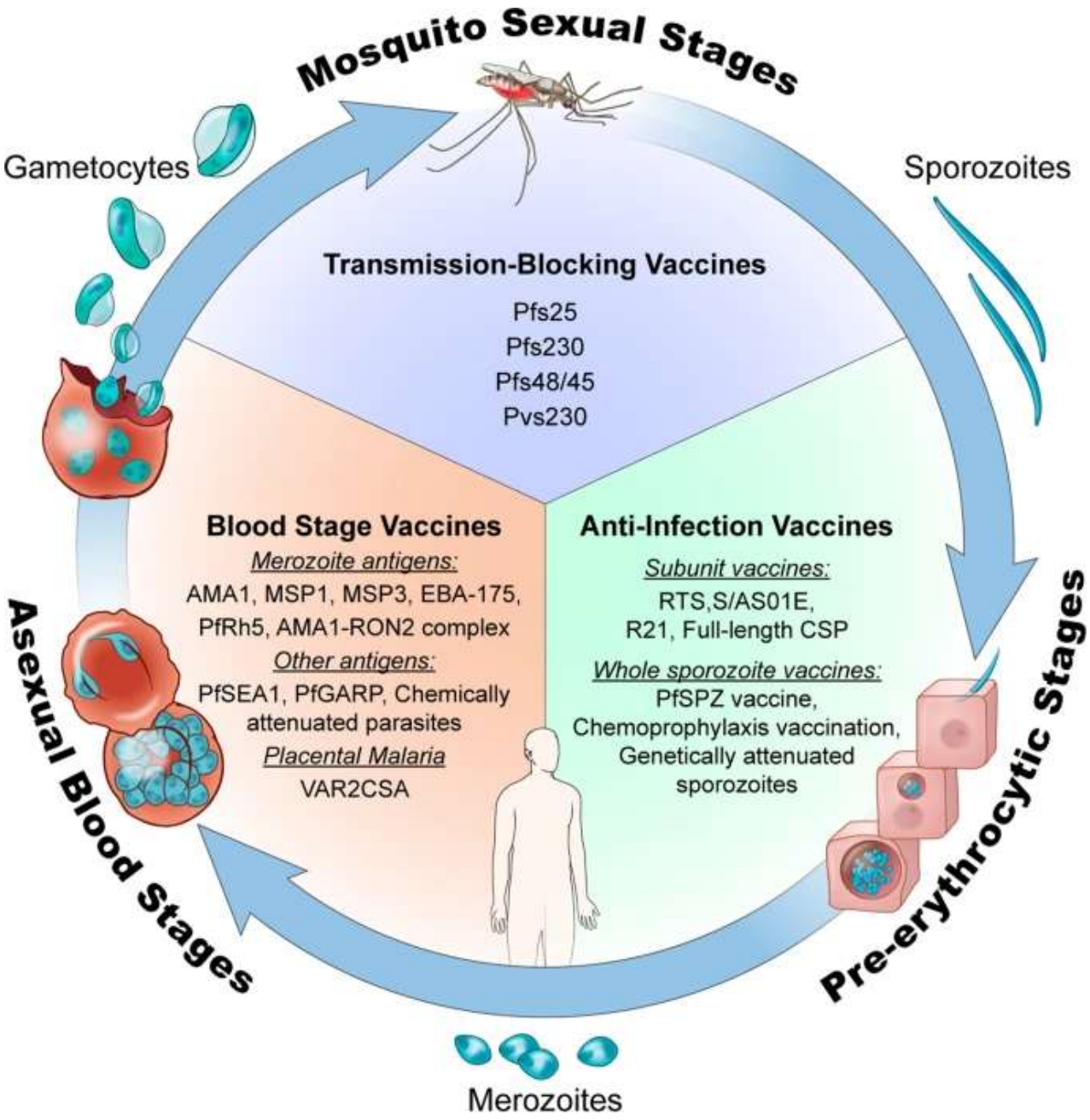

Figure 2: Lifecycle stages of *Plasmodium* and vaccine candidates that target each stage. Figure reproduced from a review by Duffy and Gorres, 2020 [32].

3.4 Rationale for a transmission-blocking vaccine against *P. falciparum* malaria

Transmission-blocking vaccines (TBVs) aim to induce antibodies against sexual-stage antigens, which are taken up in the blood meal and act to prevent sexual development in the mosquito host, thus blocking onward malaria transmission. Uniquely, TBVs do not aim to provide protection against infection or disease in the vaccinated individual but instead to reduce risk of infection at the population level, through interruption of the transmission cycle.

The first observations of transmission-blocking immunity (TBI) were reported in 1958, when lower infectivity to mosquitoes of the avian *Plasmodium* species, *P. fallax* and *P. gallinaceum*, were described in turkeys and chickens which had been immunised with formalin-killed parasitised erythrocytes [33]. However, it was not until almost 20 years later, in 1976, that the phenomenon was reproduced and the underlying mechanism delineated as antibody-mediated inhibition of

sexual parasite development in the mosquito midgut [34]. In 1983, following developments in methods for *in vitro* culture of the sexual stages of *P. falciparum*, BALB/c mice were immunised with *P. falciparum* gametes leading to the generation of the first transmission-blocking monoclonal antibodies (mAbs), which could arrest parasite development in the mosquito midgut [35, 36]. Through surface radio-isotope labelling of gametes followed by precipitation with these transmission-blocking mAbs, the first list of transmission-blocking targets were identified [36].

The gold standard *ex vivo* model for establishing transmission-reducing (conferring decreased oocyst intensity) or transmission-blocking (reduction in mosquito infection prevalence) effects, is the standard membrane feeding assay (SMFA), wherein mosquitoes are fed cultured gametocytes in the presence of whole serum or purified IgG, from either a test or control source. Mosquitoes are then dissected approximately a week later and oocyst development in the mosquito is quantified by microscopic visualisation using mercury-bromide staining, and compared between intervention and control groups. For determination of TBI against varied parasitic strains, direct-membrane feeding assays (DMFAs), using gametocytes derived from field isolates, may also be employed. Vaccine-induced antibodies generated against the TBV candidate antigens first described in 1983, have reproducibly demonstrated capacity to reduce *P. falciparum*, since their identification [36-38].

3.5 Challenges and directions in *P. falciparum* transmission-blocking vaccine development

The TBV candidates first identified in 1983, Pfs25, Pfs48/45 and Pfs230, named due to their molecular mass, remain the most well-studied and developed vaccine candidates to date [36-38]. The Pfs25 paralogue, Pfs28, was later identified as a further target mediator of TBI. Pfs28, structurally and genetically similar to Pfs25, has demonstrated a synergistic effect alongside Pfs25 pre-clinically [39], although this was not reproduced in a similar study investigating the *P. vivax* equivalent, Pvs28 [40]. Pfs48/45 is highly important in male fertility, with knockout studies resulting in male gametes that are incapable of adhering to and penetrating female gametes, with consequent significant reductions in oocyst development [41]. Pfs230 has an important role in the interaction of exflagellated microgametes with adjacent red blood cells for formation of exflagellation centres and subsequent oocyst development. However, knockout studies for both *pfs48* and *pfs230* genes suggest that their function is non-essential, since disrupted strains display highly reduced oocyst formation, but not complete lack of development [41, 42].

More recently, PfHAP2, essential for fusion of the male and female gametocyte during fertilization, has also been demonstrated to induce transmission-blocking in the murine model [43]. In addition, in a novel approach, mosquito-derived antigens are under investigation to target immune responses to prevent parasite penetration of the mosquito midgut [44, 45]. However, these candidates, alongside other potential new transmission-blocking targets of interest, remain at early pre-clinical stages of investigation.

Classical transmission-blocking candidates can be considered to fall into two broad categories: those that target antigens which are expressed before fertilisation and those that are only expressed in the post-fertilisation stages within the mosquito. Pfs48/45 and Pfs230 are the principal pre-fertilisation targets, whilst Pfs25 is the leading post-fertilisation candidate, along with Pfs28 [37]. Naturally-acquired immunity to pre-fertilisation antigens, which are present in the human host, have been identified in sero-epidemiological studies in The Gambia, Papua New Guinea and Cameroon, including anti-Pfs230 and anti-Pfs48/45 antibody responses, which correlated with transmission-reducing activity (TRA) [46-54]. This suggests that vaccine-induced responses against these targets may benefit from boosting in natural infection. Conversely, repression of translation prior to development in the mosquito vector means that post-fertilisation

antigens do not come under immune pressure, resulting in very limited polymorphism. Polymorphisms in the pre-fertilisation antigens are more frequent, although are still less common than in blood-stage antigens [55].

The induction of sustained, high-titre antigen-specific antibody responses, maintained over a minimum of a transmission season, will be critical for an efficacious TBV. Data from an inter-species comparative assessment of Pfs25-specific IgG antibody from mouse, rabbit, monkey and human sera also suggest that higher human antibody titres are required to produce transmission-blocking effects relative to animal models used in pre-clinical studies, with human IgG producing a 50% reduction in oocyst intensity by SMFA (IC50) at an estimated concentration of 85.6 μg/mL compared to 15.9 μg/mL, 4.2 μg/mL, 41.2 μg/mL for mice, rabbit and monkey sera, respectively [56]. The requirement for high human antibody titres for effecting TRA was reproduced in a clinical trial of the Pfs25-EPA/Alhydrogel vaccine candidate, in which the IC50 was estimated as 57.2 μg/ml (95% CI 44.7, 76.8) in sera from vaccinated subjects [57].

Further to the development of vaccines developed specifically targeting transmission stages, vaccines candidates targeting the liver or blood-stages of malaria infection, if effective, may also have a role in reducing transmission, by preventing blood-stage infection and therefore gametocyte development entirely, or efficient blood-stage parasite clearance. The modest and short-lived efficacy demonstrated by the most advanced malaria vaccine candidate, RTS,S, may limit the impact on malaria transmission of this pre-erythrocytic vaccine alone [28, 29]. However, the combination of multiple antigens, targeting different stages has the potential to provide synergistic effects to enhance vaccine efficacy. Pre-clinical studies evaluating combinations of TBVs with vaccines targeting other life-cycle stages, or TBI as mediated by monoclonal antibodies (mAb), in combination with mAbs against the sporozoite stage circumsporozoite protein have demonstrated promising results [58, 59]. Additional investigation of this multi-stage strategy will likely be an important investigational approach in the development of an effective malaria vaccine for malaria control and reduced parasite transmission.

3.6 Pfs48/45 as an antigen

Pfs48/45 is a cysteine motif-rich protein expressed during *P. falciparum* gametocyte maturation. These gametocytes display multi-protein complexes (MPCs) on the plasma membrane, comprised of the PfCCp protein family of six secreted proteins. The PfCCp-based MPCs are linked to the gametocyte plasma membrane via interactions with Pfs230, a binding-partner of the glycophosphatidylinositol (GPI)-anchored Pfs48/45 (Figure 2). This MPC assembly step is a crucial step for sexual reproduction of the parasite [60].

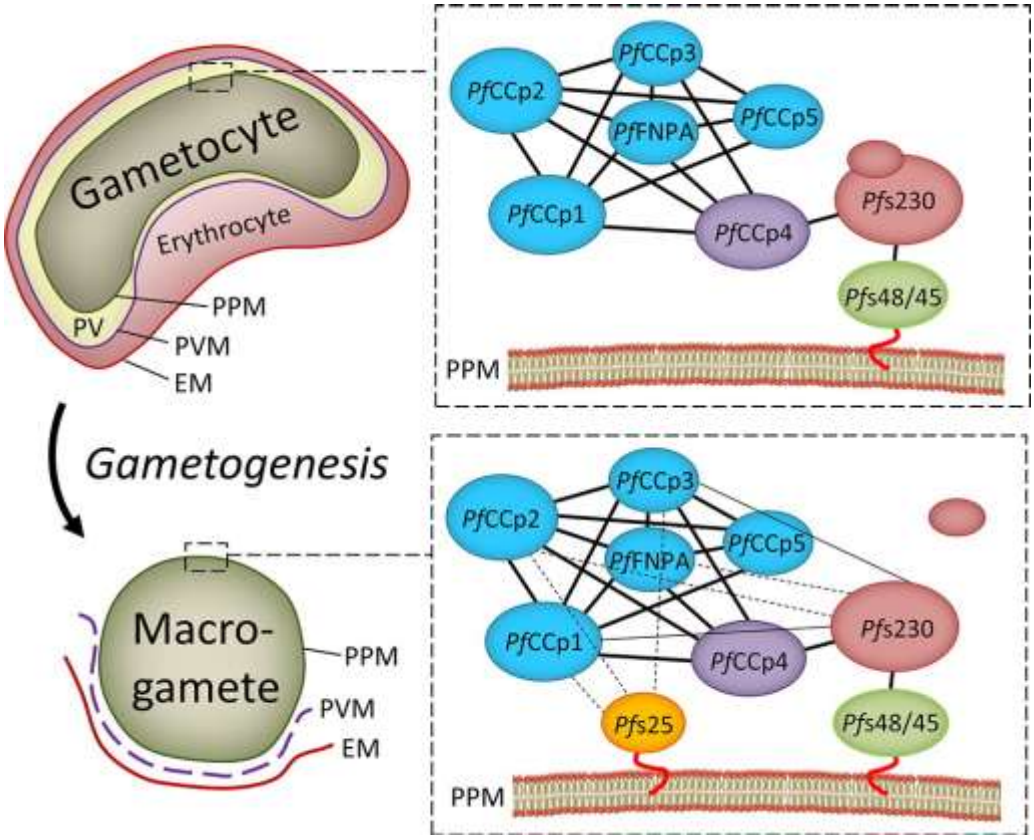

Figure 3: Schematic representation of the *Plasmodium falciparum* multi-protein complex (MPC) composition and assembly during gametogenesis. Processing of Pfs230 during gametogenesis is represented by the release of a protein fragment. EM = erythrocyte membrane; PPM = parasite plasma membrane; PV = parasitophorous vacuole; PVM = parasitophorous vacuole membrane. Figure reproduced from Simon *et al*, 2015 [60].

A major bottleneck to Pfs48/45 vaccine development is the challenge of producing the properly folded recombinant protein in sufficiently high yields. As Pfs48/45 is cysteine-rich, correct formation of disulfide bridges is essential. This refolding into the native structure is required for the induction of transmission-blocking antibodies.

Pfs48/45 has been produced in different eukaryotic expression systems including baculovirus (Sf9) cells [61], vaccinia virus [62], *Saccharomyces cervesiae* [63], *Pichia pastoris* [63], *Chlamydomonas reinhardtii* [64], *Escherichia coli* [65-68] and *Nicotiana benthamiana* [69]. However, these systems have led to disappointing results with low yields of properly folded recombinant proteins or minimal reactivity with monoclonal antibodies against conformational transmission-blocking epitopes. In contrast, the *Lactococcus lactis* expression system has been more successful, with the R0.10C [70-72] and R0.6C [73] proteins demonstrating good yields, correct folding and functional activity.

Several lines of evidence from pre-clinical studies suggest Pfs48/45 as a good TBV candidate. For instance, studies demonstrate that monocolonal antibodies directed against Pfs48/45 lead to prevention of parasite transmission to mosquitoes in SMFAs [36, 74]. In addition, antibody-prevalence studies in endemic areas show that natural antibodies against Pfs48/45 correlate with TRA [75]. Furthermore, several rodent studies show that properly folded fragments of Pfs48/45 are able to elicit high levels of transmission-blocking antibodies in the host [65, 70, 73, 76].

3.7 Clinical trials of Pfs48/45-based vaccines

Only one Pfs48/45-based vaccine has reached clinical trials. R0.6C is a multistage chimera consisting of a transmission blocking (Pfs48/45-6C) and a blood-stage (GLURP-R0) antigen. In pre-clinical studies using the *Lactoccus lactis* expression system, the GLURP-R0 antigen facilitated synthesis of a correctly folded Pfs48/45 C-terminal fragment [70]. Then, a series of novel fusion proteins were generated and tested in rats with aluminium hydroxide as an adjuvant [73]. Functional transmission-blocking antibodies were generated with transmission-blocking activity associated with specific IgG levels [73].

The safety and efficacy of this R0.6C vaccine is currently being tested in the Netherlands in healthy adults as a Phase I clinical trial. The STOP-TRANS study [NCT04862416] commenced recruitment in May 2021 and aims to recruit 32 participants across four groups. The single domain R0.6C vaccine will be adjuvanted with Alhydrogel alone, or combined with Matrix-M.

Of note is the fact that R0.6C consists of just a single domain of Pfs48/45 whereas the vaccine we are studying in this proposed trial contains all three domains of the protein. Transmission blocking epitopes have been found within all three domains of Pfs48/45, hence a full length Pfs48/45 vaccine offers more targets to elicit transmission blocking antibodies against, compared to R0.6C. [65, 76-79].

4 PFS48/45 IN MATRIX-M VACCINE DEVELOPMENT

4.1 Characteristics of Pfs48/45 in Matrix-M

Pfs48/45 is a soluble protein vaccine based on the *P. falciparum* transmission-blocking target antigen. The Pfs48/45 protein is based on the sequence from the 3D7 *P. falciparum* strain. The recombinant protein is expressed in, and secreted from, the insect Schneider 2 (S2) cell line derived from *Melanogaster drosophila*.

Matrix-M is a potent, saponin-based adjuvant, comprising defined purified fractions from bark extracts of the bark of the *Quillaja saponaria* Molina tree, phosphatidylcholine and cholesterol, formulated as a 40nm-sized complex. Matrix-M adjuvant has been shown to efficiently activate and recruit immune cells to the draining lymph node, including T-cells, B-cells, NK-cells, dendritic cells and granulocytes, which may lead to enhanced antigen presentation [80]. Matrix-M has demonstrated a favourable safety profile, as well as the enhancement of cellular and humoral immune responses to a range of vaccine [80-82].

Pfs48/45 and Matrix-M adjuvant will be mixed immediately prior to administration for co-administration.

4.2 Vaccine development strategy

The clinical development of Pfs48/45 in Matrix-M adjuvant is aimed towards the production of an effective transmission-blocking malaria vaccine for individuals in malaria endemic regions. This vaccine would be used in a three-dose vaccination regimen (Table 1).

Group	Group size	D0	D28	D56
Group 1	8-10	Pfs48/45 10 µg Matrix-M 50 µg	Pfs48/45 10 µg Matrix-M 50 µg	Pfs48/45 10 µg Matrix-M 50 µg
Group 2	8-10	Pfs48/45 50 µg Matrix-M 50 µg	Pfs48/45 50 µg Matrix-M 50 µg	Pfs48/45 50 µg Matrix-M 50 µg
Group 3	8-10	Pfs48/45 50 µg Matrix-M 50 µg	Pfs48/45 50 µg Matrix-M 50 µg	Pfs48/45 10 µg Matrix-M 50 µg

Table 1: Vaccination protocol for study groups 1, 2 and 3

This first-in-human, dose-escalation study aims to provide an initial assessment of the frequency and magnitude of AEs following vaccination with Pfs48/45 in the Matrix-M adjuvant for evaluation of safety, as well as immunogenicity and *ex vivo* efficacy via membrane feeding assays. In addition, the optimal dose of Pfs48/45 in Matrix-M1 will be determined from this trial. Data generated from this study will inform progression to a Phase Ib trial in an endemic region to evaluate the safety and immunogenicity of Pfs48/45 in Matrix-M at various doses, in a similar dose-escalation study, to establish the optimal dose and schedule in an endemic setting.

4.3 Pfs48/45 immunogenicity and *ex vivo* efficacy in pre-clinical studies

The Pfs48/45 soluble protein vaccine being studied in this proposed trial is the full-length protein consisting of all three protein domains. In pre-clinical studies involving mice, the immunogenicity of different proteins (as measured by anti-Pfs48/45 IgG titres) was assessed by ELISA. Here, three 5 µg doses of different forms of the protein was administered to each group three weeks apart with AddaVax as an adjuvant. Figure 4 shows that mice immunised with both versions of the full-length protein had high levels of anti-Pfs48/45 IgG titres.

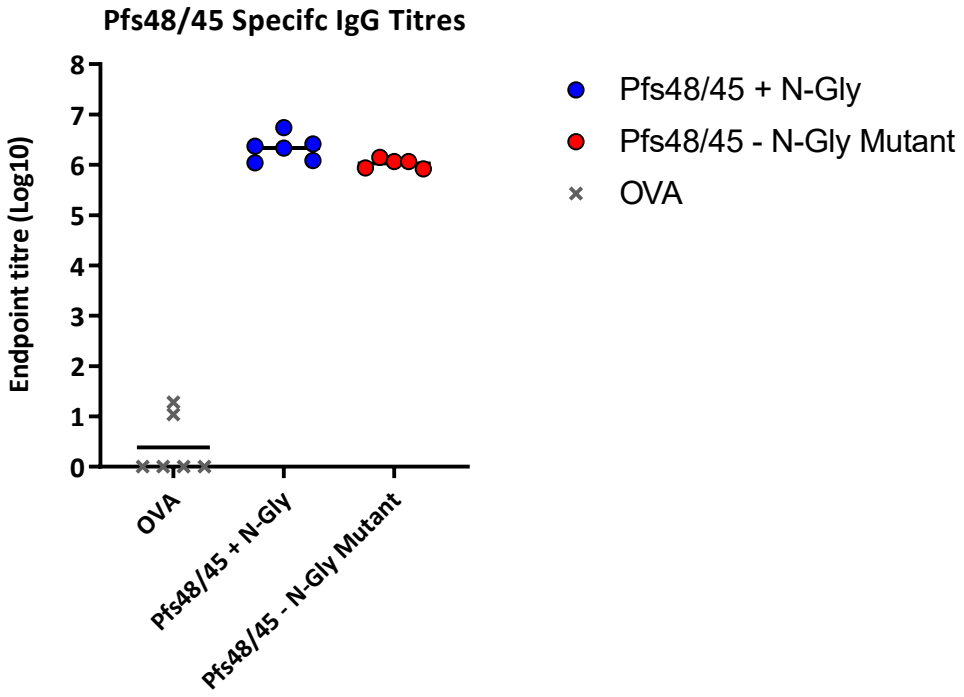

Figure 4: Anti-Pfs48/45 IgG titres in mice immunised with different forms of the Pfs48/45 protein. CD1 outbred mice were immunised with three 5 µg doses of vaccine three weeks apart with AddaVax as an adjuvant and IgG titres measured at two weeks following the third dose. The following protein were assessed: Full-length Pfs48/45 protein (AA: 27-428) with all seven N-glycans sites (Pfs48/45 + N-Gly), full-length Pfs48/45 protein with all seven N-glycans sites mutated out (Pfs48/45 – N-Gly Mutant). OVA=ovalbumin (negative control).

In addition, SMFA showed that full-length Pfs48/45 induces transmission reducing activity (TRA) when IgG antibodies from immunised mice was diluted into blood containing stage V NF54 gametocytes and fed to 3-6 day old starved female *Anopheles stephensi* mosquitoes (Figure 5). Here, there were decreased oocyst numbers per mosquito when mice were immunised with full-length Pfs48/45 compared with controls.

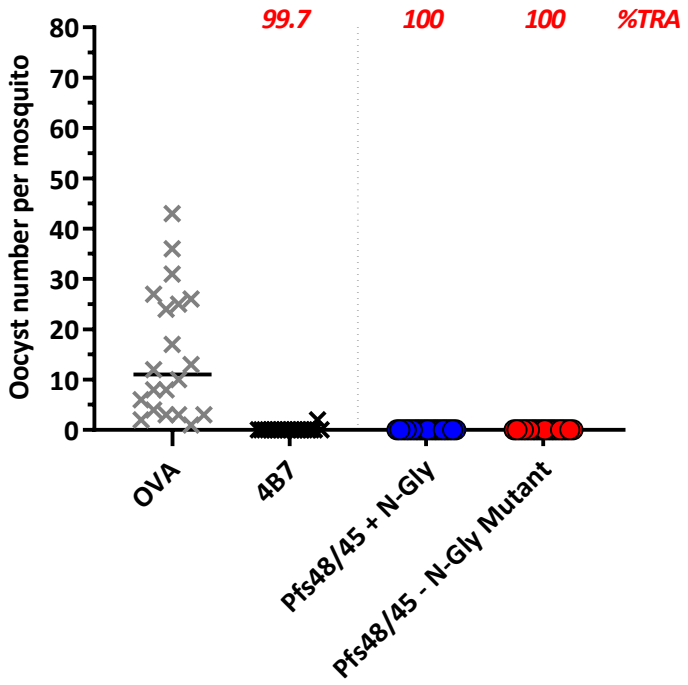

Figure 5: Standard membrane feeding assay (SMFA) involving mice immunized with different forms of the Pfs48/45 protein at a concentration of 750 μg/ml of purified IgG. Red numbers above each column denote percentage transmission reducing activity (%TRA) for each protein. OVA = ovalbumin (negative control); 4B7 = Pfs25 specific mouse control monoclonal antibody (positive control); Pfs48/45 + N-Gly = full-length Pfs48/45 with all seven N-glycans sites present; s48/45-FL – N-Gly Mutant = full-length Pfs48/45 with all seven N-glycans sites absent.

Finally, data suggests that the combination of Pfs48/45 with Matrix-M resulted in higher immunogenicity in mice when compared to Pfs48/45 with Alhydrogel (Figure 6). Here, CD1 outbred mice were immunised with two doses of 0.1 μg or 1 μg Pfs48/45 three weeks apart with Alhydrogel or Matrix-M. They were bled at three weeks following the second dose and immunogenicity assessed by anti-Pfs48/45 IgG ELISA and SMFA. The ELISA data suggests that the Matrix-M adjuvant potentiated increased levels of anti-Pfs48/45 IgG titres when compared to the Alhydrogel adjuvant, with minimal difference between the 0.1 μg and 1 μg dose.

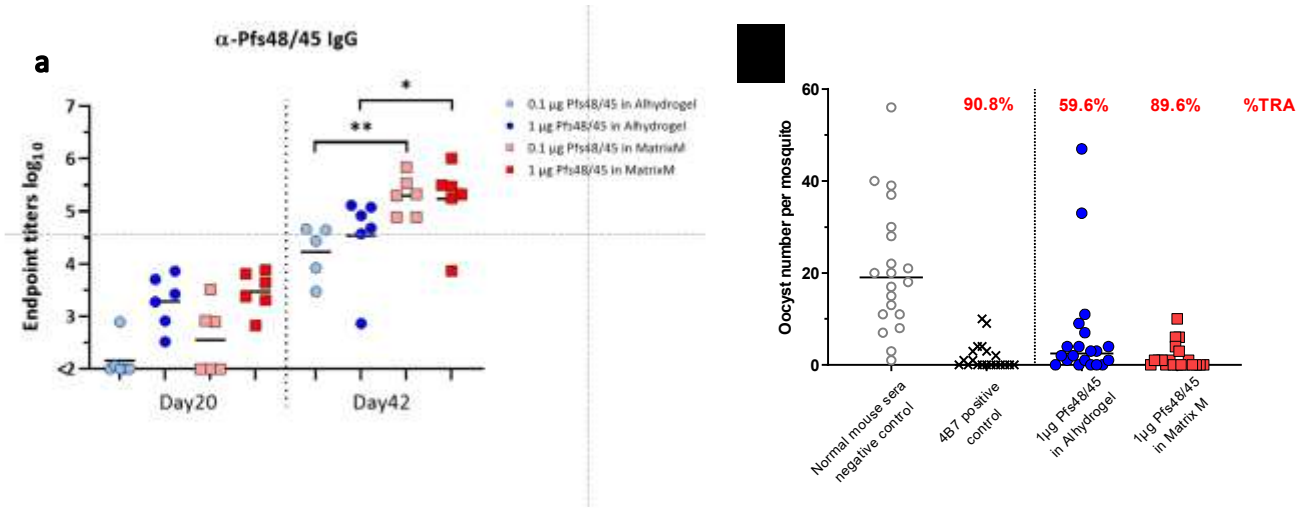

Figure 6: Comparison of Alhydrogel and Matrix-M as adjuvants for immunisation with Pfs48/45. CD1 outbred mice were immunized with either two doses of 0.1 µg or 1 µg of Pfs48/45 with either adjuvant at three-week intervals and bled at day 20 and day 42. A: Pfs48/45 specific IgG endpoint titres of immunized mice. B: SMFA of IgG purified from D42 of immunized mice. Normal mouse sera acts as a negative control and the Pfs25 specific mAb 4B7 as positive control.

4.4 Clinical experience with Pfs48/45 in Matrix-M

There is no prior clinical experience with the Pfs48/45 protein specifically in the Matrix-M adjuvant.

Matrix-M adjuvant, has been administered to tens of thousands of individuals in clinical trials across the USA, Europe, Africa and Australia. In addition to trials involving vaccines to respiratory syncytial virus, influenza and SARS-CoV-2, two malaria pre-erythrocytic candidate vaccines (ChAd63/MVA ME-TRAP and VLP R21) have been administered with Matrix-M adjuvant in four phase I and phase I/II trials conducted in Oxford and Burkina Faso (ClinicalTrials.gov identifiers: NCT02572388, NCT02925403, NCT01669512, NCT02905019). A phase III trial involving 4800 children has recently started in Africa with sites in Burkina Faso, Mali, Kenya and Tanzania. Available safety data demonstrates that the Matrix-M adjuvant is well-tolerated with acceptable short-term reactogenicity and unremarkable long-term safety profile in COVID-19 vaccine clinical trials conducted by Novavax collaborators in subjects who received Matrix-M [83].

The profile of AEs following vaccination with Pfs48/45 may be partially predicted from previous studies assessing protein vaccines using the Matrix-M adjuvant. Local AEs are likely to include injection site pain, erythema, swelling, itching and warmth. Forseeable systemic AEs would include headache, fatigue, myalgia, arthralgia, malaise, feverishness, fever and nausea.

5 INVESTIGATIONAL PRODUCTS

5.1 Pfs48/45

5.1.1 Vaccine supply

Pfs48/45 is manufactured by GenIbet (Oeiras, Portugal), where the vaccines are formulated and vialed. Xerimis Ltd (Reading, UK) will perform certification and QP release, labelling and storage of the Pfs48/45 for investigational use only, and later supply to the clinical site (CCVTM).

5.1.2 Vaccine formulation and packaging

The Pfs48/45 protein drug product is formulated in 20 mM Tris Buffered Saline (TBS), 150mM NaCl, pH 7.4 (adjusted by sodium hydroxide). The final 0.2 µm filtered drug product was dispensed manually using an electronic dispenser preset to deliver 500 µl into type 1, 2 ml glass vials. Filling is performed by Apotex Production and Laboratory. The vials were sterilized and depyrogenated with a 13 mm grey bromobutyl rubber stopper supplied by Adelphi Healthcare Packaging (Haywards Heath, UK) and a 13 mm complete tear off, clear lacquered aluminium seal. The containers and closures were tested for compliance with defined specifications.

5.1.3 Storage of Vaccines

All vaccines will be stored at -80°C (nominal). All movements of the study vaccines will be documented. Vaccine accountability, storage, shipment and handling will be in accordance with relevant local Standard Operating Procedures (SOPs) and forms.

5.2 Matrix-M

5.2.1 Matrix-M adjuvant supply

Matrix-M adjuvant will be supplied to Xerimis by Novavax AB (Uppsala, Sweden), a subsidiary of Novavax, Inc (Gaithersburg, USA), where the adjuvant is formulated and vialed. Matrix-M will be labelled for investigational use only by Xermis Ltd before supplying the adjuvant to the clinical site.

All adjuvants will be certified for release by a qualified person (QP) at Xerimis Ltd.

5.2.2 Matrix-M adjuvant formulation and packaging

Matrix-M is formulated at a concentration of 0.375mg/ml in PBS. The drug product is filled into sterile 3 mL glass vials. Matrix-M adjuvant is a slightly brown to colourless, slightly opalescent, non-viscous liquid.

5.2.3 Matrix-M adjuvant storage and Handling

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

5.3 Administration of Pfs48/45 in Matrix-M adjuvant

A mixture of Pfs48/45 at a dose of 10 µg or 50 µg, with 50 µg of Matrix-M adjuvant will be administered to all volunteers. Matrix-M and Pfs48/45 will be mixed immediately prior to administration and will be administered intramuscularly within one hour of thawing of Pfs48/45.

All vaccines will be administered intramuscularly in the deltoid muscle of the non-dominant arm preferentially, according to SOP VC002 Vaccination. The vaccinating investigator will wear gloves

and eye protection. During administration of the vaccines, Advanced Life Support (ALS) drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. On vaccination day, vaccines will be allowed to thaw to room temperature and administered within one hour. After insertion of the needle but before injection, aspiration should be attempted to ensure that the injection is not intravascular. A single vial of vaccine will be used per dose administered and any remaining vaccine not used will be discarded.

6 OBJECTIVES AND ENDPOINTS

6.1 Primary Objectives

To assess safety and tolerability of the Pfs48/45 with Matrix-M vaccine at various doses in healthy adult volunteers.

6.1.1 Primary Outcome Measures

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

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

Solicited and unsolicited AE data will be collected at each clinic visit. It will be collected from e-diaries, clinical review, clinical examination (including observations) and laboratory results. This AE data will be tabulated, detailing frequency, duration and severity of AEs.

Haematological and biochemical laboratory values will be presented according to local grading scales. SAEs, AEs of special interest and withdrawal due to AE(s)/SAE(s) will be described in detail. Volunteers will be followed for approximately 8 months following initial dose of Pfs48/45 with Matrix-M and approximately 6 months following the third dose.

6.2 Secondary Objectives

To assess the humoral and cellular immunogenicity of the Pfs48/45 with Matrix-M vaccine, when administered to healthy adult volunteers.

To assess *ex vivo* efficacy of the Pfs48/45 with Matrix-M vaccine, when administered to healthy adult volunteers.

6.2.1 Secondary Immunological Outcome Measures

Pfs48/45-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 Pfs48/45 protein – total IgG, isotypes and avidity; and T cell responses to Pfs48/45 by *ex vivo* enzyme-linked immunospot assay (ELISpot).

Ex vivo functional blocking activity of purified IgG against *P. falciparum* will be assessed by membrane feeding assays .

Other established and exploratory immunology assays may be carried out, which may include collaboration with other specialist laboratories within or beyond Europe. This would involve transfer of serum/plasma, but samples would be anonymised. Volunteers will be consented for this.

The secondary timepoints will be days 1, 29, 57, 140 and 240.

7 DESCRIPTION AND JUSTIFICATION OF STUDY DESIGN

7.1 Study rationale

As outlined above, the currently available methods for preventing and treating malaria remain inadequate. Although focused efforts have led to substantial reductions in morbidity and mortality worldwide, progress has now stalled. An effective vaccine against *P. falciparum* would contribute substantially to disease control and elimination efforts.

Pre-clinical studies suggest Pfs48/45 as a good TBV candidate. In rodent studies, properly folded fragments of Pfs48/45 are able to elicit high levels of transmission-blocking antibodies in the host [65, 70, 73, 76, 84]. The Pfs48/45 protein we are testing in this study differs from previously studied versions of the antigen in that the vaccine candidate being tested is full-length, offering a wider variety of blocking epitopes than the 6C-based counterparts. In addition, combining Pfs48/45 with the Matrix-M adjuvant, which has been shown to be an effective adjuvant in combination with a range of different vaccines, offers a promising strategy.

This small, first-in-human study will provide initial data on the safety profile of vaccination with Pfs48/45 in the Matrix-M adjuvant, as well as immunogenicity and *ex vivo* efficacy via membrane feeding assays.

7.2 Rationale for selected doses

The two doses of 10 µg and 50 µg Pfs48/45 in 50 µg Matrix-M were selected on the basis of experience with other candidate protein vaccines which have been tested at the same dose in humans with the same adjuvant. These include the VAC077 and VAC082 trial of Pfs25-IMX313 in Matrix-M1 as well as the VAC084 trial of Pvs25-IMX313 in Matrix-M1.

Mouse studies suggest that a vaccine dose of up to 250 µg/kg is safe. In this study we are testing a maximum dose of 50 µg Pfs48/45 in 50 µg Matrix-M. This is equivalent to a dose of 0.8 µg/kg in humans. This means that there is a safety margin based on body weight of 250/0.8 = 312-fold in humans as compared to mice.

7.3 Study overview

This is an open-label, single-centre, non-randomised, first-in-human Phase Ia study to assess the safety and immunogenicity of the Pfs48/45 vaccine, administered with Matrix-M adjuvant.

Up to 30 healthy, malaria-naïve adults aged between 18 and 45 years will be recruited into one of three groups (n=8-10 per group) at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Oxford over approximately 12 months.

All volunteers will receive three doses of Pfs48/45 at 10 µg or 50 µg, in 50 µg Matrix-M, administered intramuscularly and given four weeks apart. Enrolment will be staggered. Initially, a single volunteer from Group 1 will receive the first vaccination alone, followed by clinical review on the day after vaccination and further monitoring via a diary card for at least a further 24 hours. Provided there are no safety concerns, a further two volunteers from Group 1 may then be vaccinated, at least 1 hour apart. Following review of the second and third volunteers at the 48-hour timepoint, an internal safety review will be conducted. If no safety concerns are identified, the remaining volunteers in the group will be vaccinated. The staggered enrolment process will be repeated for Group 2 for the first three volunteers. Following this, recruitment to Groups 2 and 3 will occur in parallel.

Volunteers will attend regular follow-up visits after each of the three vaccinations and will self-report AEs in the post-vaccination period via a diary card. Blood will be taken at regular intervals to assess safety outcomes via laboratory adverse events, as well as the immune response to vaccination. Volunteers will be followed until approximately 8 months from the time of enrolment.

All vaccinations and follow-up visits will be performed at the CCVTM in Oxford.

7.4 Study groups

There will be three study groups (Group 1, Group 2 and Group 3), each comprising 8-10 volunteers. There will be staggered enrolment for Groups 1 and 2 as described below in Section 7.3.1. Any volunteer who is withdrawn or withdraws from the study may be replaced at the discretion of the Investigators.

7.4.1 First volunteers

The first volunteer from Group 1 to receive Pfs48/45 in Matrix-M will be enrolled and vaccinated alone. Providing there are no safety concerns at 48 hours after the first volunteer has been vaccinated, the second and third volunteers from Group 1 will be vaccinated. Following a further 48 hour period, an internal safety review will be conducted involving the review of participant e-diaries by the Chief Investigator with the Lead Clinician. If at this point there are no safety concerns, the rest of Group 1 may be immunised. This staggered approach will be repeated for the second dose. After at least eight volunteers from Group 1 have received two out of three doses of 10 µg Pfs48/45 in 50 µg Matrix-M and had seven days of follow-up, there will be a formal safety review (see below) prior to dose escalation (i.e. recruitment to Groups 2 and 3). The staggered enrolment process will continue for the third vaccination in Group 1 and for Group 2 for the first three volunteers at each of the three doses. Following this, recruitment to Groups 2 and 3 will occur in parallel.

7.4.2 Dose escalation

A minimum of data from eight volunteers recruited to Group 1 will be reviewed prior to dose escalation (i.e. recruitment to Groups 2 or 3). The data to be reviewed will include but is not limited to: physical observations, patient e-diary cards and safety bloods (full blood count, urea and electrolytes, liver function tests). Data up to and including the first seven days following vaccination will be required for each of the eight volunteers prior to dose escalation. E-diary cards will be reviewed on a daily basis for the first eight volunteers recruited to Group 1 for the first seven days following vaccination. A formal safety review will be undertaken by the LSM prior to dose escalation. This would take the form of a safety report incorporating the relevant safety data described above, which would need to be reviewed and approved by the CI and LSM prior to recruitment into Groups 2 and 3.

7.4.3 Group 1

Eight to ten volunteers will be recruited into Group 1 (Table 1) and each volunteer will attend for three vaccinations. On the day of enrolment, Pfs48/45 will be administered at a dose of 10 µg in 50 µg Matrix-M adjuvant. After approximately 4 weeks, a second dose will be administered, followed by a third and final vaccination approximately 4 weeks later. Second and third vaccinations will be administered at the same dose of both vaccine and adjuvant as at the initial vaccination. Volunteers will be followed until 8 months (approximately) from the time of enrolment.

7.4.4 Group 2

Eight to ten volunteers will be recruited into Group 2 (Table 1) and each volunteer will attend for three vaccinations. On the day of enrolment, Pfs48/45 will be administered at a dose of 50 µg in 50 µg Matrix-M adjuvant. After approximately 4 weeks, a second dose will be administered, followed by a third and final vaccination approximately 4 weeks later. Second and third vaccinations will be administered at the same dose of both vaccine and adjuvant as at the initial vaccination. Volunteers will be followed until 8 months (approximately) from the time of enrolment.

7.4.5 Group 3

Eight to ten volunteers will be recruited into Group 3 (Table 1) and each volunteer will attend for three vaccinations. On the day of enrolment, Pfs48/45 will be administered at a dose of 50 µg in 50 µg Matrix-M adjuvant. After approximately 4 weeks, a second dose will be administered, followed by a third and final vaccination approximately 4 weeks later. The second vaccination will be at the same dose as the first vaccination whilst the third vaccination will be administered at a lower dose of Pfs48/45 10 µg in 50 µg Matrix-M adjuvant. Volunteers will be followed until 8 months (approximately) from the time of enrolment.

7.5 Duration of study

7.5.1 Definition of the start and end of the trial

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

7.5.2 Duration of volunteer participation

Volunteers will be considered to be enrolled in the trial on the receipt of the first vaccination. Total duration of follow-up will be approximately 8 months from enrolment.

7.6 Potential risks for volunteers

7.6.1 Phlebotomy

The maximum estimated volume of blood drawn (942mL) over the study period should not compromise otherwise healthy volunteers, however, volunteers will be monitored for anaemia. There may be minor bruising, local tenderness, pre-syncopal symptoms or syncope (rarely) associated with venepuncture, which will not be documented as AEs if they occur. The actual total blood volume may vary depending on any requirement for repeat clinical tests, e.g. in the event of a clinically significant abnormal result.

7.6.2 Vaccinations

Potential foreseeable risks from vaccination can be partially predicted from previous studies assessing protein vaccines, administered with the Matrix-M adjuvant, which include local and systemic reactions. Generally, an increase in solicited AEs is seen following administration of investigational vaccines with Matrix-M adjuvant, compared to the antigen alone or placebo, however, reactogenicity is transient with the vast majority of adverse events resolving within 2-7 days.

Local AEs are likely to include injection site pain, erythema, swelling, itching and warmth. Forseeable systemic adverse events would include: headache, fatigue, myalgia, arthralgia, malaise,

feverishness, fever and nausea. The majority of local and systemic AEs are foreseen to be mild to moderate in nature, however, it is anticipated that rarely, these may be reported as severe. Of the systemic AEs reported as severe following receipt of other investigational vaccines administered with Matrix-M adjuvant, myalgia is the most common.

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

7.7 Potential benefits for volunteers

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

8 RECRUITMENT AND WITHDRAWAL FOR TRIAL VOLUNTEERS

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

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

8.1 Informed consent

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

- Participation in the study is entirely voluntary.
- Refusal to participate involves no penalty or loss of medical benefits.
- The volunteer may withdraw from the study at any time.
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved.
- There is no direct benefit from participating.
- The volunteer's general practitioner (GP) will be contacted to corroborate their medical history. Volunteers will only be enrolled in the study if written information regarding the volunteer's medical history is obtained from the GP.
- The volunteer will be registered on the TOPS database (The Overvolunteering Prevention System; www.tops.org.uk).
- With volunteer consent, blood samples will be taken and any leftover samples stored indefinitely for use in other, ethically approved research.

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial and will then have time to consider whether or not to participate before completing the written consent form. In addition to the consent form, volunteers will be asked to complete an additional separate form regarding consent for the GP to release medical records, a copy of which will be sent to the GP.

The participant must personally sign and date the latest approved version of the Informed Consent Form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information Sheet and Informed Consent Form will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the trial. Written informed consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent.

The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. A copy of the signed Informed Consent Form will be given to the participant. The original signed form will be retained at the trial site.

8.2 Inclusion and exclusion criteria

8.2.1 Inclusion criteria

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

- Healthy adult aged 18 to 45 years.
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Willing to allow the Investigators to discuss the volunteer's medical history with their GP.
- Volunteers with the potential to become pregnant only: must practice continuous effective contraception for the duration of the study (see section 10.10).
- Agreement to refrain from blood donation for the duration of the study.
- Able and willing to provide written informed consent to participate in the trial.

8.2.2 Exclusion criteria

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

- History of clinical malaria (any species).
- Travel to a clearly malaria endemic locality during the study period or within the preceding six months.
- Use of immunoglobulins or blood products (e.g., blood transfusion) in the last three months.
- Receipt of any vaccine in the 30 days preceding enrolment, or planned receipt of any other vaccine within 30 days following each study vaccination, with the exception of COVID-19 vaccines, which should not be received between 14 days before to 7 days after any study vaccination.
- Receipt of an investigational product in the 30 days preceding enrolment, or planned receipt during the study period.
- Concurrent involvement in another clinical trial or planned involvement during the study period.
- Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data, as assessed by the Investigator.
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days)

immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed).
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine.
- Any history of anaphylaxis in reaction to vaccinations.
- Pregnancy, lactation or intention to become pregnant during the study.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of serious psychiatric condition that may affect participation in the study.
- Any other serious chronic illness requiring hospital specialist supervision.
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 25 standard UK units every week.
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Hepatitis B surface antigen (HBsAg) detected in serum.
- Seropositive for hepatitis C virus (antibodies to HCV) at screening (***unless*** volunteer has taken part in a prior hepatitis C vaccine study with confirmed negative HCV antibodies prior to participation in that study, and negative HCV ribonucleic acid (RNA) PCR at screening for this study).
- Volunteers unable to be closely followed for social, geographic or psychological reasons.
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination. In the event of abnormal test results, confirmatory repeat tests will be requested. Procedures for identifying laboratory values meeting exclusion criteria are shown in SOP VC027.
- Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.
- Inability of the study team to contact the volunteer's GP to confirm medical history

8.2.3 Prevention of 'Over Volunteering'

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

8.2.4 Vaccination and re-vaccination exclusion criteria

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

- Anaphylactic reaction following administration of vaccine.
- Pregnancy.

The following AEs constitute contraindications to administration of vaccine at that point in time; if any one of these AEs occurs at the time scheduled for vaccination, the participant may be vaccinated at a later date, or withdrawn at the discretion of the Investigator. The participant must be followed until resolution of the event as with any adverse event:

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to

persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C/99.5°F.
- Temperature of >37.5°C (99.5°F) at the time of vaccination.
- Current COVID-19 infection, defined as ongoing symptoms with positive COVID-19 PCR swab test taken during current illness or positive COVID-19 PCR swab or rapid antigen test within preceding 7 days without symptoms. Vaccinations will be delayed by a minimum of 7 days from the date of the first positive COVID-19 PCR swab or rapid antigen test, as long as symptoms are improving or resolved. It will be at the discretion of the Investigator to withdraw a participant if they develop severe COVID-19 disease.

8.2.5 Concomitant medications

As set out by the exclusion criteria, volunteers may not enter the study if they have received any vaccine in the 30 days prior to enrolment or there is planned receipt of any other vaccine within 30 days of each vaccination, any investigational medicinal product within 30 days prior to enrolment or if receipt is planned during the study period, or any chronic (more than 14 days) immunosuppressant medication within 6 months prior to enrolment or if receipt is planned at any time during the study period (inhaled and topical steroids are permitted). One exception is COVID-19 vaccines, which should not be received between 14 days before to 7 days after any study vaccinations.

8.3 Withdrawal of volunteers

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

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

The Local Safety Monitor (LSM) may recommend withdrawal of volunteers. The reason for withdrawal from further study procedures will be recorded in the Case Report Form (CRF) where available. For all AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

Any volunteer who fails to attend for two or more follow-up visits will be deemed to have withdrawn from the study.

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

In all cases of participant withdrawal, excepting those of complete consent withdrawal, long-term safety data collection, including some procedures such as safety bloods, will continue as appropriate if participants have received one or more vaccine doses.

8.4 Pregnancy

Should a volunteer become pregnant during the trial, they will be followed up as other volunteers and in addition will be followed until pregnancy outcome, with their permission. Depending on the timings of pregnancy outcome relative to planned study follow-up timepoints, this may involve additional telephone consultations. We will not routinely perform venepuncture on such volunteers.

9. TREATMENT OF TRIAL VOLUNTEERS

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

9.1 Trial sites

Volunteers will be recruited and undergo all visits at the CCVTM, Oxford, this is inclusive of screening, vaccinations and follow-up visits.

9.2 Study procedures

Procedures will be performed at the time points indicated in the schedule of events (Table 2). Additional procedures or laboratory tests may be performed, at the discretion of the Investigators if clinically necessary.

9.2.1 Observations

Pulse, blood pressure and temperature will be measured at the time points indicated in the schedule of events (Table 2). In addition, weight and height will be measured at screening.

9.2.2 Blood tests

Blood will be drawn at the time points indicated in the schedule of events (Table 2) and the following laboratory assays performed:

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

- **Haematology:** Full blood count.
- **Biochemistry:** Sodium, potassium, urea, creatinine, albumin and liver function tests.
- **Diagnostic serology:** HBsAg, HCV antibodies, HIV antibodies.
- **Immunology:** Human Leukocyte Antigen (HLA) typing at the day of enrolment (D0).

At the Jenner Institute research laboratories:

- **Immunology**:
    - Pfs48/45-specific immunogenicity will be assessed by a variety of immunological assays. These will include *ex vivo* ELISpot assays for interferon gamma and antibody ELISAs for measuring total IgG, isotype and avidity.
    - The functional activity of the vaccine-induced antibodies will be tested using membrane-feeding assays. The functional activity of the purified IgG will be tested against *P. falciparum* by membrane feeding assay.
    - Other exploratory immunological assays including cytokine analysis, other antibody assays (plasma cell and memory B cell assays), DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity and gene expression studies, amongst others, may be performed at the discretion of the Investigators. B cells, plasma and/or serum may also be analysed and used to produce human monoclonal antibodies.
    - Samples may also be sent to collaborating laboratories within and outside the UK (including outside of Europe) for other immunological assays as required. This would involve the transfer of serum, plasma or blood cells to these laboratories, but these would remain pseudo-anonymised. Informed consent for this will be obtained from

volunteers. Immunological assays will be conducted according to the procedures established in the test laboratories.

With the volunteers' informed consent, any leftover cells, plasma, serum (or their purified components) will be stored indefinitely in the Oxford Vaccine Centre Biobank for future immunological analysis of malaria-specific or vaccine-related responses. This may include human DNA and RNA analysis. If a participant elects not to permit this, all of that participant's leftover samples will be discarded after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

9.2.3 Urinalysis

Urine will be tested for the presence of clinically significant proteinuria, glycosuria or haematuria (as defined in Appendix A) at the screening visits. For volunteers with the potential to become pregnant (see section 10.10 for definition), urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening, immediately prior to each vaccination, at days 0, 28 and 56 and on day 84.

9.2.4 Vaccinations

Before each vaccination, the ongoing eligibility of the volunteer will be reviewed. The vaccine will be administered as described above in section 5.3. The injection site will be covered with a sterile dressing and the volunteer will stay in the local trial site for observation for 1 hour, in case of immediate AEs. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes after vaccination (+/- 10 minutes).

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

9.2.5 Management of post-vaccination fevers

Post-vaccination fevers may cause diagnostic uncertainty as concurrent COVID-19 infection is a possibility. If a participant develops a fever within the first 24 hours after vaccination and no other symptoms associated with COVID-19 disease (new onset cough, anosmia or ageusia), then post-vaccination fever is most likely.

If a participant develops a fever post-vaccination, which is associated with either new onset cough or anosmia or ageusia, they will be advised to self-isolate as per UK Health Security Agency (UK HSA) guidance and directed to local community based COVID-19 testing. Whilst awaiting their COVID-19 PCR test results, participants will be advised not to attend the clinic for study visits.

If a participant tests positive for COVID-19 at any stage of the study, they will be advised to follow current UK HSA guidance in place. During clinic visits protective equipment (PPE) will be worn as per the infection control SOP that covers all PPE requirements for clinical trials at the Oxford Vaccine Centre in the era of the COVID-19 pandemic.

Any study visits which are due whilst a participant is self-isolating, should be rescheduled to occur when self-isolation ends, if this falls within the visit window. If a participant remains in self-isolation during the period of the visit window, then the study visit will be conducted by telephone and no blood tests will be taken. Study visit windows have been increased in order to accommodate these scenarios.

If a participant is self-isolating for a prolonged period post-vaccination and hence would miss all three initial post vaccination visits, then it will be at the Investigator's discretion to conduct these as physical visits at CCVTM to allow full assessment including blood tests. These study visits would be conducted in a side room with study staff wearing level 1 PPE.

9.2.6 E-diary

Following each vaccination, volunteers will be asked to complete an e-diary. Volunteers will be asked about foreseeable local and systemic AEs for 7 days (solicited AEs). After this, volunteers will be asked to record any AE daily, for 28 days (unsolicited AEs). E-diaries will be reviewed with volunteers at each post-vaccination clinic visit.

An electronic PDF copy of the e-diary or hard copy will be issued to participants if there are technical issues with the e-diary or if the participant has internet/accessibility issues.

9.2.7 Photographs

Photographs of the injection site may be taken in the event of severe pain, redness and/or swelling following vaccination to enable assessment of any improvement/deterioration. Severe is defined as a grading of 3 in any one of the categories detailed in Table 6. Photographs will not include the participant's face.

9.3 Study visits

All clinical reviews and procedures will be undertaken by one of the clinical team. The procedures to be included in each visit are documented in Table 2. Each review is assigned a time point and a window period within which the review will be conducted.

9.3.1 Screening visits

There will be an initial pre-screening questionnaire that is completed online (https://www.onlinesurveys.ac.uk) to assess eligibility. Use of this tool has been approved by the University of Oxford. If the prospective participant meets initial eligibility criteria, we will seek consent via email to subsequently contact individuals by telephone. During this pre-screening telephone call with a member of the clinical team to discuss the study, we will provide the opportunity for interested individuals to discuss the study, eligibility criteria and study requirements. If individuals continue to express an interest in taking part in the trial following the pre-screening call, and if they meet eligibility criteria, a screening visit will be scheduled.

Screening visits may take place up to 90 days prior to enrolment. Informed consent will be gained at screening. If consent is given, the screening procedures indicated in the schedule of events (Table 2) will be undertaken.

The potential participant's GP will be contacted with the written permission of the participant after screening using the GP screening letter. This will enable the study team to ascertain any significant medical history and act as notification that the participant has volunteered for the study.

During the screening the volunteers will be asked to provide their National Insurance or passport number so that this can be entered on to a national database which helps prevent volunteers from participating in more than one clinical trial simultaneously or over-volunteering for clinical trials (https://www.tops.org.uk).

Where more than 90 days has lapsed between the initial screening visit and date of enrolment, a re-screening visit will be conducted to ascertain if there have been any changes in the medical history or physical examination findings. Urinalysis, biochemistry and haematology blood tests will

be repeated. Other blood tests will not be repeated routinely. Where a reply from the potential participant's GP has already been received, the GP will not be re-contacted unless there is any need to obtain futher information where appropriate (e.g. regarding a new medical event or finding) and at the discretion of the Investigator.

Hepatitis B, Hepatitis C and HIV status will be checked via blood tests at the screening visit. Some individuals may test positive for Hepatitis C virus due to their previous involvement in a Hepatitis C vaccine study. If this applies to an individual and they wish to take part in our study, with their consent, we will contact the team responsible for the Hepatitis C vaccine study to check the participant's Hepatitis C status prior to enrolment in this malaria vaccine study. A copy of the consent will be held by both study teams.

9.3.2 Enrolment and first vaccination (V1) with Pfs48/45 in Matrix-M adjuvant (all groups)

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

At the day 0 visit, any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β-hCG test in volunteers with the potential to become pregnant and venepuncture for immunology and safety bloods will be undertaken according to Table 3. The inclusion and exclusion criteria for the study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the Pfs48/45 vaccine, formulated in Matrix-M adjuvant will be administered according to the doses described in Table 2 and as described in Section 5.3. The visit will be carried out as described in above Section 9.2.4.

9.3.3 Reviews post-vaccination on days 1, 7 and 14 (all groups)

On subsequent visits, the volunteers will be assessed for local and systemic AEs, using the e-diary, interim history, physical examination and blood tests at the time points indicated in the schedule of events (Table 2). Blood will also be taken for exploratory immunology analyses.

Volunteers will be asked to record all AEs via the e-diary. E-diaries will be reviewed with volunteers at the clinic visits on days 1 and 7. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). They will also be asked daily about any new symptoms from day 7 until day 28 via the diary but will not be asked directly about foreseeable symptoms. E-diaries will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or previously undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 2. At any post-vaccination visit, where valid written, informed consent is in place, photographs may be taken for clinical comparison. This will enable qualitative as well as quantitative assessment of redness and swelling at the injection site. Confidentiality will be maintained as described in Section 13.5.

9.3.4 Second vaccination (V2) with Pfs48/45 in Matrix-M adjuvant on day 28 (all groups)

This visit will include a follow-up visit for the first vaccination and administration of the second vaccine. Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β-hCG test in volunteers with the potential to become pregnant and venepuncture for immunology and safety bloods will be undertaken according to Table 2. The inclusion and exclusion criteria for the study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the Pfs48/45 vaccine formulated in Matrix-M adjuvant will be administered intramuscularly and preferentially into the opposite arm (if

possible)to that used for the first vaccination and as described in sections 5.3 and 9.2.4. Dosing will be determined by group allocation (see Table 1).

9.3.5 Reviews post-vaccination on days 29, 35 and 42 (all groups)

On subsequent visits, the volunteers will be assessed for local and systemic AEs, using the e-diary, interim history, physical examination and blood tests at the time points indicated in the schedule of events (Table 2). Blood will also be taken for exploratory immunology analysis.

Volunteers will be asked to record all AEs via the e-diary. E-diaries will be reviewed with volunteers at the clinic visits on days 29 and 35. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). They will also be asked daily about any new symptoms from day 35 until day 56 via the diary but will not be asked directly about foreseeable symptoms. E-diaries will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 2. At any post-vaccination visit, where valid written, informed consent is in place, photographs may be taken for clinical comparison. Confidentiality will be maintained as described in Section 13.5.

9.3.6 Third vaccination (V3) with Pfs48/45 in Matrix-M adjuvant on day 56 (all groups)

This visit will include a follow-up visit for the second vaccination and administration of the third vaccine. Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β-hCG test in volunteers with the potential to become pregnant and venepuncture for immunology and safety bloods will be undertaken according to Table 2. The inclusion and exclusion criteria for the study will be reviewed. If the volunteer remains eligible and there are no contraindications to vaccination, the Pfs48/45 vaccine, formulated in Matrix-M adjuvant will be administered intramuscularly and preferentially into the opposite arm (if possible) to that used for the second vaccination and as described in sections 5.3 and 9.2.4. Dosing will be determined by group allocation (see Table 1).

9.3.7 Reviews post-vaccination on days 57, 63, 70 and 84

On subsequent visits, the volunteers will be assessed for local and systemic adverse events, using the e-diary, interim history, physical examination and blood tests at the time points indicated in the schedule of attendances (Table 2). Blood will also be taken for exploratory immunology analysis.

Volunteers will be asked to record all AEs via the e-diary and at visits on days 57 and 63, during which e-diaries will be reviewed with volunteers. Volunteers will be asked directly about foreseeable local and systemic AEs for 7 days (solicited AEs). Volunteers will be asked to continue to record any new symptoms from day 63 until day 84, on a daily basis via the diary but will not be asked directly about foreseeable symptoms. E-diaries will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations, procedures (including a urinary pregnancy test at D84) and venepuncture for immunology and safety bloods will be undertaken according to Table 3. At any post-vaccination visit, where valid written, informed consent is in place, photographs may be taken for clinical comparison. Confidentiality will be maintained as described in Section 13.5.

9.3.8 Long-term follow up post-vaccination on days 140 and 240

On days 140 and 240, volunteers will be assessed for serious adverse events, by interim history and physical examination, as deemed necessary by the Investigator. Blood tests will be performed as indicated in the schedule of attendances for these timepoints (Table 2). Blood will also be taken for exploratory immunology analysis. At any post-vaccination visit, where valid written, informed consent is in place, photographs may be taken for clinical comparison. Confidentiality will be maintained as described in Section 13.5.

	S	V1			V2				V3							
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Timeline (days)		0	1	7	14	28	29	35	42	56	57	63	70	84	140	240
Window (days)	-90		±1	±2	-2/+4	-7/+14	±1	±2	-2/+4	-7/+14	±1	±2	-2/+4	-7/+14	±14	±14
Inclusion/Exclusion criteria	X	X														
Informed consent	X	X														
Physical observations*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history/ examination	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Photographs		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urinalysis	X															
Urine β-hCG**	X	X				X				X				X		
Review contraindications		X				X				X						
Review AEs & medications			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaccination		X				X				X						
Diary card issued		X				X				X						
Diary card collected						X				X				X		
Immunology plasma (mL)		40	10	10	30	30	10	60	30	30	10	60	110	80	30	30
Immunology serum (mL)		40			20	40			40	40			30	40	20	20
RNA preservation (mL)		3	3			3	3			3	3					
Haematology (mL)[#]	2	2		2	2	2		2	2	2		2	2	2		
Biochemistry (mL) [##]	3	3		3	3	3		3	3	3		3	3	3		
HLA typing (mL)		4														
HIV, HBV, HCV serology (mL)	5															
Total Blood Volume (mL)	10	92	13	15	55	78	13	65	75	78	13	65	145	125	50	50
Cumulative blood volume (mL)	10	102	115	130	185	263	276	341	416	494	507	572	717	842	892	942[$]

Table 2: Schedule of events

***S** = screening visit; **V =** vaccination**; (X)** = If considered necessary, emphasising any acute complaints. ***Physical observations will include blood pressure, heart rate and temperature, plus height and weight at screening only. ****For volunteers with the potential to become pregnant. **#** Haematology will comprise full blood count. **##** Biochemistry will include sodium, potassium, urea, creatinine, albumin, liver function tests. **$** Cumulative blood volume if blood taken as per schedule and excluding any repeat safety blood tests that may be necessary.*

10 ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of AEs and SAEs arising during the study. The safety profile will be assessed on an ongoing basis by the Investigators during the safety reporting window for each participant (from provision of consent to their last study visit). The CI and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

10.1 Definitions

10.1.1 Adverse Event (AE)

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

Each participant-reported AE will be graded by the participant according to the table for grading severity of adverse events (see Table 3). Severity gradings may be reviewed and discussed with the participants at the clinic visits.

10.1.2 Adverse Reaction (AR)

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

10.1.3 Serious Adverse Event (SAE)

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

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

- Congenital anomaly or birth defect.

10.1.4 Serious Adverse Reaction (SAR)

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

10.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

All SAEs at least possibly related to Pfs48/45 in Matrix-M will be considered unexpected and be reported as SUSARs.

10.1.6 Foreseeable Adverse Reactions

The foreseeable ARs following vaccination with Pfs48/45 in Matrix-M adjuvant are: injection site pain, erythema, warmth, swelling, pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea. These AEs will be listed as 'solicited AEs' providing they occur within 7 days of the day of vaccination. 'Unsolicited AEs' are AEs other than the foreseeable ARs occurring within the first 7 days, or any AEs occurring after the first 7 days after vaccination.

10.2 Causality Assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI or the CI-delegated clinician at the coordinating site (Oxford). An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy (Table 3).

Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, for which causality should be assigned by the clinically trained reporting Investigator.

0	**No Relationship**	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
1	**Unlikely**	Unlikely temporal relationship to study product ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to study product.

2	**Possible**	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
3	**Probable**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
4	**Definite**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

Table 3: Guidelines for assessing the relationship of vaccine administration to an AE.

10.3 Reporting Procedures for all AEs

All AEs occurring in the 28 days following each vaccination observed by the Investigator or reported by the volunteer, whether or not attributed to the study vaccine, will be recorded. Recording and reporting of all AEs will take place as detailed in SOP VC027. We will not record bruising due to blood-taking or cannulation. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). SAEs will be collected throughout the entire trial period. For each participant, this means the safety reporting window per participant (from provision of study consent to final follow-up visit).

Each AE will be graded by the participant according to the table for grading severity of AEs (see Table 4). Severity gradings may be reviewed and discussed with the participants at the clinic visits. Volunteers will be instructed on how to self-assess the severity of these AEs. There will also be space on the e-diary to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

The severity of clinical AEs will be assessed by the Investigators according to the scales in Tables 4-6. Severity grading for laboratory AEs are dependent on the Oxford University Hospitals laboratory's reference and will be graded according to the scales in the site-specific table that can be found in the Trial Master File (TMF). These ranges will be based on FDA guidance relative to local laboratory reference ranges [https://www.fda.gov/media/73679/download].

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

Table 4: Severity grading criteria for AEs.

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

Table 5: Severity grading criteria for clinically significant abnormal physical observations. All observations should be measured at rest. *Only applies when resting heart rate is between 60 and 100 beats per minute. Use clinical judgement when characterising bradycardia in some healthy participant populations (e.g. conditioned athletes). **Systolic or diastolic hypertension may only be confirmed as clinically significant (and therefore an AE) if persistently present when observations are repeated (i.e. isolated measurements of hypertension are not clinically significant).

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity

Erythema at injection site	1	>3 - ≤50 mm
	2	>50 - ≤100 mm
	3	>100 mm
Swelling at injection site*	1	>3 - ≤20 mm
	2	>20 - ≤50 mm
	3	>50 mm

Table 6: Severity grading criteria for local adverse events. *Erythema or swelling ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event.

10.4 Reporting Procedures for SAEs

This will be conducted in accordance with the SOP OVC005 Safety Reporting. In order to comply with current regulations on SAE reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported on the SAE forms to members of the study team immediately after the Investigators become aware of their occurrence, as described in SOP OVC005. Copies of all reports will be forwarded for review to the CI (as the Sponsor's representative) within 24 hours of the Investigator being aware of the suspected SAE. The Local Safety Monitor (LSM) will be notified of SAEs which are deemed possibly, probably or definitely related to study interventions; the LSM will be notified immediately (within 24 hours) of the Investigators' being aware of their occurrence. SAEs will not normally be reported immediately to the REC unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, at the discretion of the CI and/or LSM. In addition to the reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

10.4.1 Reporting Procedures for SUSARS

The CI will report all SUSARs to the MHRA and REC within required timelines (15 days for all SUSARs, unless life-threatening or fatal. Fatal and life-threatening SUSARs will be reported to the MHRA and REC no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days of awareness. The CI will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

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

10.5 Development Safety Update Report

The CI will submit (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK, Ethics Committee, HRA, Host NHS Trust and Sponsor).

10.6 AEs of Special Interest

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

- Severe hypersensitivity reactions (e.g. anaphylaxis)
- Any new, suspected autoimmune or chronic inflammatory disease

10.7 Procedures to be followed in the event of abnormal findings

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

10.8 Local Safety Monitor

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

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

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

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

10.9 Safety Stopping/Holding Rules

Safety holding rules have been developed considering the fact that this is a first-in-human study.

10.9.1 Group holding rules (applies to Groups 1 and 2/3)

If a holding rule is activated, then further vaccinations in the group (Group 1 and/or Group 2/3) will not occur until an internal safety review has been conducted and it is deemed appropriate to restart dosing. Note that as Groups 2 and 3 will be recruited in parallel, if a group holding rule is activated for one of the two groups, then further vaccinations in both groups would go on hold. The regulatory authority must be informed of the hold via substantial amendment and a request

to restart dosing with pertinent data must be submitted as a further, subsequent substantial amendment. The internal safety review will consider:

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

The local ethics committee and adjuvant manufacturers (Novavax) will also be notified if a holding rule is activated or released to ensure that each Party is obliged to comply with the performance and activities related to the clinical trial (there is a confidential Safety Data Exchange Agreement, SDEA, in place). All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs. Novaxax will be provided with SAE and pregnancy reports and any safety concerns causing a suspension as per the SDEA beween Oxford and Novavax

The group holding rules are as follows:

- **Solicited local AEs:**
    - If two or more doses of a vaccine are followed by a Grade 3 solicited local AE beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 72 hrs.

- **Solicited systemic AEs:**
    - If two or more doses of a vaccine are followed by a Grade 3 solicited systemic AE beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 48 hrs.

- **Unsolicited AEs:**
    - If two or more volunteers develop a Grade 3 unsolicited AE (including the same laboratory AE) that is considered possibly, probably or definitely related to vaccination.

    - However, if a study participant has a Grade 3 unsolicited (or laboratory) AE considered 'possibly' related to vaccination which persists at Grade 3 < 48 hours that, in the opinion of the Investigator, is of non-clinical significance and where a different cause is judged as likely, the event will not be counted as part of the group holding AEs.

- **A serious AE considered possibly, probably or definitely related to vaccination occurs**

10.9.2 Individual stopping rules

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

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

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

- **Solicited systemic AEs:**

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

- **Unsolicited AEs:**

    - The volunteer has a Grade 3 AE, considered possibly, probably or definitely related to vaccination, persisting continuously at Grade 3 for >72hrs.

    - The volunteer has an acute allergic reaction or anaphylactic shock following the administration of the vaccine investigational product.

- **A serious AE considered possibly, probably or definitely related to vaccination occurs**

10.10 Contraception and pregnancy

Volunteers with the potential to become pregnant are defined as those who are fertile and able to become pregnant following menarche until becoming post-menopausal, unless permanently sterile. A post-menopausal state is defined as no menstruation for 12 months without a known cause.

Volunteers with the potential to become pregnant are required to use an effective form of contraception during the course of the study as this is a Phase I, first-in-human, study and there is currently no information about the effect of this vaccine on a foetus.

Acceptable forms of contraception for volunteers with the potential to become pregnant are:

- Established use of oral, injected or implanted hormonal methods of contraception.
- Placement of an intrauterine device or intrauterine system.
- Male sterilization: if the vasectomised partner is the sole partner for the participant.
- True abstinence from sex with sperm-producing partners, when this is in line with the preferred and usual lifestyle of the participant (periodic abstinence and withdrawal are not acceptable methods of contraception).

Pregnancy, lactation or intention to become pregnant during the study is an exclusion criteria.

11 STATISTICS

11.1 Sample size

This is an observational and descriptive safety study, where healthy adult volunteers living in the UK will be vaccinated with Pfs48/45, in formulation with the Matrix-M adjuvant. Up to a total of 30 volunteers across three equally divided groups will be vaccinated. Each volunteer will receive three doses of Pfs48/45, formulated in 50 µg Matrix-M, on days 0, 28 and 56 (see Table 2 for exact dosing regimen depending on group allocation). This sample size should allow estimation and comparison of the frequency and magnitude of the outcome measures, at and between the three different dosing regimens. As this is the first study involving the Pfs48/45 in Matrix-M vaccine, there is no available data to enable more precise modelling/power calculations. However, it is considered that eight to ten volunteers in each group will be sufficient to enable detection of statistically significant differences in the primary and secondary outcome measures (see Section 6) between the groups. Specifically, we will be able to assess the optimal dose for future studies based on the safety/tolerability as well as immunogenicity of each of the three dosing regimens.

12 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

12.1 Investigator procedures

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

12.2 Monitoring

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

12.3 Modification to protocol

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

An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the participant's safety, the objectives of the trial and its progress. An administrative change may not require UK ethical committee approval; however, the REC will be notified in the event of any such change.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which ethical committee(s) approval has already been given, are not initiated without review and approval from the ethics committee(s) and regulatory authorities except to eliminate apparent immediate hazards to the participant (Urgent Safety Measures only). Protocol adherence is a fundamental part of the conduct of a clinical study. Changes to the approved protocol need prior approval unless for urgent safety reasons. Investigators must not request a protocol waiver to enter a patient who does not satisfy the selection criteria.

12.4 Protocol deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the TMF.

12.5 Audit & inspection

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

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

12.6 Serious breaches

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

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

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

(b) the scientific value of the trial.

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

12.7 Publication policy

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

12.8 Intellectual Property

Ownership of intellectual property generated by employees of the University vests in the University. The protection and exploitation of any new intellectual property is managed by the University’s technology transfer office, Oxford University Innovation.

13 ETHICS

13.1 Declaration of Helsinki

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

13.2 GCP guidelines

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

13.3 Approvals

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

13.4 Reporting

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

13.5 Volunteer confidentiality

The research data will be pseudoanonymised; participant data will be identified by a unique study number in the CRF and database. Separate confidential files containing identifiable information and links to the study number will be stored in secured locations. These secured locations are either physical premises or web servers owned by the University of Oxford or in the case of the eCRF on the OpenClinica database. The OpenClinica database has undergone the Third Party Security Assessment process and been officially approved as a processor of data for which the University of Oxford is the controller. Only the Sponsor, Investigators, the clinical monitor, the ethical committee(s) and the regulatory authorities will have access to the records.

Photographs taken (if required, with the volunteer's written, informed consent) will not include the volunteer's face and will be identified by the volunteer's trial specific identification number only. Once developed, photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

14 DATA HANDLING AND RECORD KEEPING

14.1 Data handling

The CI will be the data custodian with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. Data will be entered into the volunteers' CRFs in a paper and/or electronic format (using the OpenClinica™ database). Electronic data will be stored on secure servers which are outsourced by OpenClinica™. OpenClinica™ meets FDA part 11B standards and is a system approved by the University of Oxford. This includes safety data, laboratory data and outcome data. Data are entered in a web browser on personal computers and then transferred to the OpenClinica Database by encrypted (https) transfer. Safety data will also be collected through an electronic diary, which is stored on a third party server (see Section 13.5).

14.2 Record keeping

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

14.3 Source data and electronic case report forms (eCRFs)

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

14.4 Data protection

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

14.5 Storage and use of data

The University of Oxford will keep identifiable information from participants collected during the study initially for 5 years after the study has finished. This will be limited to the minimum personal data necessary. In addition, we will securely store the anonymised research data and any research documents with personal information, such as consent forms, initially for 5 years after the end of the study. The need to store this information for longer, in relation to licensing of the vaccine will be reviewed every 5 years. Once the study has been completed, all documents would be archived

in a secure facility. Files will be confidentially destroyed if storage is no longer required. For effective vaccines that may be licensed, secure storage of research data may be required for at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. Financial information will be stored for seven years in line with University of Oxford financial policy.

Where specific written consent is provided, personal details may be used to contact participants about future related research. These details and clinical information will be held separately. Note that the informed consent form from this study will be retained, separately, for as long as the participant is on the database. If participants elect to take part in another study carried out by the Jenner Institute, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition.

Where specific written consent is provided, personal details may be used to contact participants about future related research. If participants elect to take part in another study carried out by the Jenner Institute, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition.

15 FINANCING AND INSURANCE

15.1 Financing

The study is funded through a grant from OptiMalVax, a European Commission Horizon 2020 funded project, as well as through internal funds from the Nuffield Department of Medicine, University of Oxford. The OptiMalVax grant aims to funds work to develop candidate multi-stage malaria vaccines through innovative trial designs in malaria-endemic areas.

15.2 Insurance

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

15.3 Compensation

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

- Screening visit £25
- Subsequent visits:
    - Travel expenses £15 per visit
    - Inconvenience of blood tests: £10 per blood donation
    - Time required for visit: £20 per hour

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

	Time in trial (approx.)	Maximum visits	Maximum volume of blood taken (mls)	Compensation
Groups 1-3	1 year	16	942	£790

Table 7: Estimated compensation amounts**.**

16 APPENDICES

Appendix A: laboratory values for exclusion

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with Investigator discretion for interpretation of results and the need for repeated or further tests. In general, volunteers will be excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory adverse event, according to the laboratory adverse event tables (filed in the TMF), on repeat of an abnormal test result. Urinalysis at screening will be assessed as per the table below:

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

Table 8: Urinalysis assessment.

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

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

17 REFERENCES

1. Organization, W.H. *Malaria*. 22/06/2021]; Available from: https://www.who.int/data/gho/data/themes/malaria.
2. Kochar, D.K., et al., *Plasmodium vivax malaria.* Emerg Infect Dis, 2005. **11**(1): p. 132-4.
3. Organisation, W.H. *World malaria report 2020*. 2020 22/06/2021]; Available from: https://www.who.int/publications/i/item/9789240015791.
4. White, N.J., et al., *Malaria.* Lancet, 2014. **383**(9918): p. 723-35.
5. WHO. *WHO Fact Sheets: Malaria*. 2021; Available from: https://www.who.int/news-room/fact-sheets/detail/malaria.
6. Cibulskis, R.E., et al., *Malaria: Global progress 2000 - 2015 and future challenges.* Infect Dis Poverty, 2016. **5**(1): p. 61.
7. WHO. *Global Technical Strategy for Malaria 2016 - 2030.* 2015 25/06/2021]; Available from: https://www.who.int/docs/default-source/documents/global-technical-strategy-for-malaria-2016-2030.pdf?sfvrsn=c82afcc_0.
8. Bhatt, S., et al., *The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015.* Nature, 2015. **526**(7572): p. 207-211.
9. Visser, T., et al., *Rapid diagnostic tests for malaria.* Bull World Health Organ, 2015. **93**(12): p. 862-6.
10. Mbonye, A.K., et al., *A Cluster Randomised Trial Introducing Rapid Diagnostic Tests into Registered Drug Shops in Uganda: Impact on Appropriate Treatment of Malaria.* PLoS One, 2015. **10**(7): p. e0129545.
11. Ansah, E.K., et al., *The impact of providing rapid diagnostic malaria tests on fever management in the private retail sector in Ghana: a cluster randomized trial.* BMJ, 2015. **350**: p. h1019.
12. Smith Paintain, L., et al., *Community health workers and stand-alone or integrated case management of malaria: a systematic literature review.* Am J Trop Med Hyg, 2014. **91**(3): p. 461-470.
13. Kayentao, K., et al., *Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis.* JAMA, 2013. **309**(6): p. 594-604.
14. Desai, M., et al., *Impact of Sulfadoxine-Pyrimethamine Resistance on Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and Preventing Low Birth Weight.* Clin Infect Dis, 2016. **62**(3): p. 323-333.
15. Cairns, M., et al., *Estimating the potential public health impact of seasonal malaria chemoprevention in African children.* Nat Commun, 2012. **3**: p. 881.
16. WHO. *Updated WHO Policy Recommendation (October 2012): Single dose Primaquine as a gaemtocytocide in Plasmodium falciparum malaria*. 2012; Available from: https://www.who.int/malaria/pq_updated_policy_recommendation_en_102012.pdf.
17. Recht, J., E.A. Ashley, and N.J. White, *Use of primaquine and glucose-6-phosphate dehydrogenase deficiency testing: Divergent policies and practices in malaria endemic countries.* PLoS Negl Trop Dis, 2018. **12**(4): p. e0006230.
18. WHO. *World malaria report 2020*. [cited 2021 22/06]; Available from: https://www.who.int/publications/i/item/9789240015791.
19. WHO. *High burden to high impact. A targeted malaria response.* 2018; Available from: https://apps.who.int/iris/bitstream/handle/10665/275868/WHO-CDS-GMP-2018.25-eng.pdf.

20. Wongsrichanalai, C. and C.H. Sibley, *Fighting drug-resistant Plasmodium falciparum: the challenge of artemisinin resistance.* Clin Microbiol Infect, 2013. **19**(10): p. 908-16.

21. WHO. *Malaria Vaccine Roadmap*. 22/06/2021]; Available from: https://www.who.int/immunization/topics/malaria/vaccine_roadmap.

22. Sinden, R.E., *A biologist's perspective on malaria vaccine development.* Hum Vaccin, 2010. **6**(1): p. 3-11.

23. Meibalan, E. and M. Marti, *Biology of Malaria Transmission.* Cold Spring Harb Perspect Med, 2017. **7**(3).

24. Sinden, R.E., *The cell biology of sexual development in plasmodium.* Parasitology, 1983. **86 (Pt 4)**: p. 7-28.

25. Joice, R., et al., *Plasmodium falciparum transmission stages accumulate in the human bone marrow.* Sci Transl Med, 2014. **6**(244): p. 244re5.

26. Billker, O., et al., *Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito.* Nature, 1998. **392**(6673): p. 289-92.

27. Centers for Disease Control and Prevention. [cited 2021 16 July]; Available from: https://www.cdc.gov/malaria/about/biology/index.html.

28. RTS S Clinical Trials Partnership, *Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial.* Lancet, 2015. **386**(9988): p. 31-45.

29. Olotu, A., et al., *Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children.* N Engl J Med, 2016. **374**(26): p. 2519-29.

30. Arevalo-Herrera, M., et al., *Protective Efficacy of Plasmodium vivax Radiation-Attenuated Sporozoites in Colombian Volunteers: A Randomized Controlled Trial.* PLoS Negl Trop Dis, 2016. **10**(10): p. e0005070.

31. mal, E.R.A.C.G.o.V., *A research agenda for malaria eradication: vaccines.* PLoS Med, 2011. **8**(1): p. e1000398.

32. Duffy, P.E. and J. Patrick Gorres, *Malaria vaccines since 2000: progress, priorities, products.* NPJ Vaccines, 2020. **5**: p. 48.

33. Huff, C.G., D.F. Marchbank, and T. Shiroishi, *Changes in infectiousness of malarial gametocytes. II. Analysis of the possible causative factors.* Exp Parasitol, 1958. **7**(4): p. 399-417.

34. Carter, R. and D.H. Chen, *Malaria transmission blocked by immunisation with gametes of the malaria parasite.* Nature, 1976. **263**(5572): p. 57-60.

35. Ponnudurai, T., et al., *The production of mature gametocytes of Plasmodium falciparum in continuous cultures of different isolates infective to mosquitoes.* Trans R Soc Trop Med Hyg, 1982. **76**(2): p. 242-50.

36. Rener, J., et al., *Target antigens of transmission-blocking immunity on gametes of plasmodium falciparum.* J Exp Med, 1983. **158**(3): p. 976-81.

37. Nikolaeva, D., S.J. Draper, and S. Biswas, *Toward the development of effective transmission-blocking vaccines for malaria.* Expert Rev Vaccines, 2015. **14**(5): p. 653-80.

38. Delves, M.J., F. Angrisano, and A.M. Blagborough, *Antimalarial Transmission-Blocking Interventions: Past, Present, and Future.* Trends Parasitol, 2018. **34**(9): p. 735-746.

39. Duffy, P.E. and D.C. Kaslow, *A novel malaria protein, Pfs28, and Pfs25 are genetically linked and synergistic as falciparum malaria transmission-blocking vaccines.* Infect Immun, 1997. **65**(3): p. 1109-13.

40. Hisaeda, H., et al., *Antibodies to Plasmodium vivax transmission-blocking vaccine candidate antigens Pvs25 and Pvs28 do not show synergism.* Vaccine, 2001. **20**(5-6): p. 763-70.

41. van Dijk, M.R., et al., *A central role for P48/45 in malaria parasite male gamete fertility.* Cell, 2001. **104**(1): p. 153-64.

42. Eksi, S., et al., *Malaria transmission-blocking antigen, Pfs230, mediates human red blood cell binding to exflagellating male parasites and oocyst production.* Mol Microbiol, 2006. **61**(4): p. 991-8.

43. Miura, K., et al., *Functional comparison of Plasmodium falciparum transmission-blocking vaccine candidates by the standard membrane-feeding assay.* Infect Immun, 2013. **81**(12): p. 4377-82.

44. Atkinson, S.C., et al., *The Anopheles-midgut APN1 structure reveals a new malaria transmission-blocking vaccine epitope.* Nat Struct Mol Biol, 2015. **22**(7): p. 532-9.

45. Niu, G., et al., *The fibrinogen-like domain of FREP1 protein is a broad-spectrum malaria transmission-blocking vaccine antigen.* J Biol Chem, 2017. **292**(28): p. 11960-11969.

46. Roeffen, W., et al., *Association between anti-Pfs48/45 reactivity and P. falciparum transmission-blocking activity in sera from Cameroon.* Parasite Immunol, 1996. **18**(2): p. 103-9.

47. Carter, R., et al., *Restricted or absent immune responses in human populations to Plasmodium falciparum gamete antigens that are targets of malaria transmission-blocking antibodies.* J Exp Med, 1989. **169**(1): p. 135-47.

48. Healer, J., et al., *Transmission-blocking immunity to Plasmodium falciparum in malaria-immune individuals is associated with antibodies to the gamete surface protein Pfs230.* Parasitology, 1999. **119 (Pt 5)**: p. 425-33.

49. Jones, S., et al., *Naturally acquired antibody responses to recombinant Pfs230 and Pfs48/45 transmission blocking vaccine candidates.* J Infect, 2015. **71**(1): p. 117-27.

50. Ouedraogo, A.L., et al., *Naturally acquired immune responses to Plasmodium falciparum sexual stage antigens Pfs48/45 and Pfs230 in an area of seasonal transmission.* Infect Immun, 2011. **79**(12): p. 4957-64.

51. Stone, W.J.R., et al., *Unravelling the immune signature of Plasmodium falciparum transmission-reducing immunity.* Nat Commun, 2018. **9**(1): p. 558.

52. Roeffen, W., et al., *Transmission blocking immunity as observed in a feeder system and serological reactivity to Pfs 48/45 and Pfs230 in field sera.* Mem Inst Oswaldo Cruz, 1994. **89 Suppl 2**: p. 13-5.

53. Bousema, J.T., et al., *Rapid onset of transmission-reducing antibodies in javanese migrants exposed to malaria in papua, indonesia.* Am J Trop Med Hyg, 2006. **74**(3): p. 425-31.

54. Bousema, T., et al., *The dynamics of naturally acquired immune responses to Plasmodium falciparum sexual stage antigens Pfs230 & Pfs48/45 in a low endemic area in Tanzania.* PLoS One, 2010. **5**(11): p. e14114.

55. Niederwieser, I., I. Felger, and H.P. Beck, *Limited polymorphism in Plasmodium falciparum sexual-stage antigens.* Am J Trop Med Hyg, 2001. **64**(1-2): p. 9-11.

56. Cheru, L., et al., *The IC(50) of anti-Pfs25 antibody in membrane-feeding assay varies among species.* Vaccine, 2010. **28**(27): p. 4423-9.

57. Talaat, K.R., et al., *Safety and Immunogenicity of Pfs25-EPA/Alhydrogel(R), a Transmission Blocking Vaccine against Plasmodium falciparum: An Open Label Study in Malaria Naive Adults.* PLoS One, 2016. **11**(10): p. e0163144.

58. Brod, F., et al., *Combination of RTS,S and Pfs25-IMX313 Induces a Functional Antibody Response Against Malaria Infection and Transmission in Mice.* Front Immunol, 2018. **9**: p. 2780.

59. Sherrard-Smith, E., et al., *Synergy in anti-malarial pre-erythrocytic and transmission-blocking antibodies is achieved by reducing parasite density.* Elife, 2018. **7**.

60. Simon, N., et al., *Adhesion protein complexes of malaria gametocytes assemble following parasite transmission to the mosquito.* Parasitol Int, 2016. **65**(1): p. 27-30.

61. Kocken, C.H., et al., *Cloning and expression of the gene coding for the transmission blocking target antigen Pfs48/45 of Plasmodium falciparum.* Mol Biochem Parasitol, 1993. **61**(1): p. 59-68.

62. Milek, R.L., et al., *Plasmodium falciparum: heterologous synthesis of the transmission-blocking vaccine candidate Pfs48/45 in recombinant vaccinia virus-infected cells.* Exp Parasitol, 1998. **90**(2): p. 165-74.

63. Milek, R.L., H.G. Stunnenberg, and R.N. Konings, *Assembly and expression of a synthetic gene encoding the antigen Pfs48/45 of the human malaria parasite Plasmodium falciparum in yeast.* Vaccine, 2000. **18**(14): p. 1402-11.

64. Jones, C.S., et al., *Heterologous expression of the C-terminal antigenic domain of the malaria vaccine candidate Pfs48/45 in the green algae Chlamydomonas reinhardtii.* Appl Microbiol Biotechnol, 2013. **97**(5): p. 1987-95.

65. Outchkourov, N.S., et al., *Correctly folded Pfs48/45 protein of Plasmodium falciparum elicits malaria transmission-blocking immunity in mice.* Proc Natl Acad Sci U S A, 2008. **105**(11): p. 4301-5.

66. Chowdhury, D.R., et al., *A potent malaria transmission blocking vaccine based on codon harmonized full length Pfs48/45 expressed in Escherichia coli.* PLoS One, 2009. **4**(7): p. e6352.

67. Milek, R.L., et al., *Immunological properties of recombinant proteins of the transmission blocking vaccine candidate, Pfs48/45, of the human malaria parasite Plasmodium falciparum produced in Escherichia coli.* Parasite Immunol, 1998. **20**(8): p. 377-85.

68. Pritsch, M., et al., *Comparison of Intranasal Outer Membrane Vesicles with Cholera Toxin and Injected MF59C.1 as Adjuvants for Malaria Transmission Blocking Antigens AnAPN1 and Pfs48/45.* J Immunol Res, 2016. **2016**: p. 3576028.

69. Prokhnevsky, A., et al., *Development of a single-replicon miniBYV vector for co-expression of heterologous proteins.* Mol Biotechnol, 2015. **57**(2): p. 101-10.

70. Theisen, M., et al., *A multi-stage malaria vaccine candidate targeting both transmission and asexual parasite life-cycle stages.* Vaccine, 2014. **32**(22): p. 2623-30.

71. Roeffen, W., et al., *Transmission-blocking activity of antibodies to Plasmodium falciparum GLURP.10C chimeric protein formulated in different adjuvants.* Malar J, 2015. **14**: p. 443.

72. Baldwin, S.L., et al., *Synthetic TLR4 agonists enhance functional antibodies and CD4+ T-cell responses against the Plasmodium falciparum GMZ2.6C multi-stage vaccine antigen.* Vaccine, 2016. **34**(19): p. 2207-15.

73. Singh, S.K., et al., *A Plasmodium falciparum 48/45 single epitope R0.6C subunit protein elicits high levels of transmission blocking antibodies.* Vaccine, 2015. **33**(16): p. 1981-6.

74. Vermeulen, A.N., et al., *Sequential expression of antigens on sexual stages of Plasmodium falciparum accessible to transmission-blocking antibodies in the mosquito.* J Exp Med, 1985. **162**(5): p. 1460-76.

75. Stone, W.J., et al., *Naturally acquired immunity to sexual stage P. falciparum parasites.* Parasitology, 2016. **143**(2): p. 187-98.

76. Lennartz, F., et al., *Structural basis for recognition of the malaria vaccine candidate Pfs48/45 by a transmission blocking antibody.* Nat Commun, 2018. **9**(1): p. 3822.

77. Roeffen, W., et al., *Plasmodium falciparum: production and characterization of rat monoclonal antibodies specific for the sexual-stage Pfs48/45 antigen.* Exp Parasitol, 2001. **97**(1): p. 45-9.

78. Targett, G.A., et al., *Plasmodium falciparum sexual stage antigens: immunogenicity and cell-mediated responses.* Immunol Lett, 1990. **25**(1-3): p. 77-81.

79. Kundu, P., et al., *Structural delineation of potent transmission-blocking epitope I on malaria antigen Pfs48/45.* Nat Commun, 2018. **9**(1): p. 4458.

80. Bengtsson, K.L., et al., *Matrix-M adjuvant enhances antibody, cellular and protective immune responses of a Zaire Ebola/Makona virus glycoprotein (GP) nanoparticle vaccine in mice.* Vaccine, 2016. **34**(16): p. 1927-35.

81. Cox, R.J., et al., *Evaluation of a virosomal H5N1 vaccine formulated with Matrix M™ adjuvant in a phase I clinical trial.* Vaccine, 2011. **29**(45): p. 8049-59.

82. Venkatraman, N., et al., *Safety and immunogenicity of heterologous prime-boost immunization with viral-vectored malaria vaccines adjuvanted with Matrix-M.* Vaccine, 2017. **35**(45): p. 6208-6217.

83. Heath, P.T., et al., *Safety and Efficacy of NVX-CoV2373 Covid-19 Vaccine.* N Engl J Med, 2021. **385**(13): p. 1172-1183.

84. Mamedov, T., et al., *A Plant-Produced in vivo deglycosylated full-length Pfs48/45 as a Transmission-Blocking Vaccine Candidate against malaria.* Sci Rep, 2019. **9**(1): p. 9868.