

Safety, Tolerability, Immunogenicity and Protective Efficacy
Against Naturally-Transmitted Malaria in Eastern Indonesia of
Two *Plasmodium falciparum* Sporozoite Vaccines, Sanaria®
PfSPZ Vaccine and Sanaria® PfSPZ-CVac: A Randomized,
Double-Blind, Placebo-Controlled Phase 2 Trial in Healthy
Indonesian Adults

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This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, applicable regulatory government agencies, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from the Sponsor.

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with the US Code of Federal Regulations (CFR), local regulations, and Good Clinical Practice (GCP) as required by the following:

- 45 CFR 46; 21 CFR 50, 21 CFR 56, 21 CFR 11, 21 CFR 812, and 21 CFR 312
- International Council for Harmonization Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) 09NOV2016; Federal Register, 1 March 2018, Vol. 83, No. 41, p. 8882-3

All individuals responsible for the design and conduct of this study have completed Human Participants Protection Training and are qualified to be conducting this research before the enrollment of any participants. CVs for all investigators and sub-investigators participating in this trial are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).

The signature on the following page constitutes approval of this protocol and the attachments, and provides the required assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable US federal regulations and (ICH E6) guidelines.

SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the attachments, and provide the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines, and the Declaration of Helsinki.

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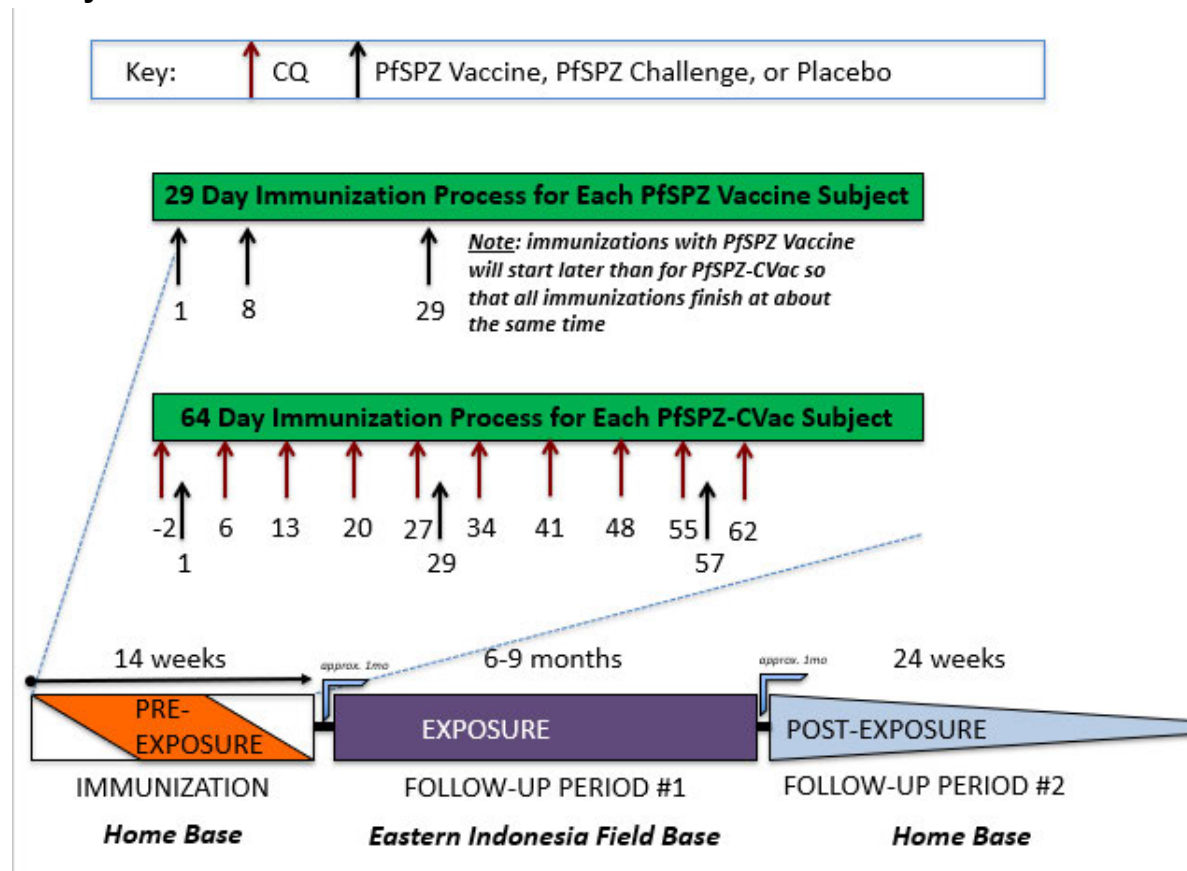
PROTOCOL SUMMARY

Title	Safety, Tolerability, Immunogenicity and Protective Efficacy Against Naturally-Transmitted Malaria in Eastern Indonesia of Two <i>Plasmodium falciparum</i> Sporozoite Vaccines, Sanaria® PfSPZ Vaccine and Sanaria® PfSPZ-CVac: A Randomized, Double-Blind, Placebo-Controlled Phase 2 Trial in Healthy Indonesian Adults
Short Title	Safety of PfSPZ Vaccine and PfSPZ-CVac and Efficacy Against Naturally Transmitted Malaria in Indonesia
Clinical Trial Phase	2
Population	Healthy males aged 18-55 years
Number of Sites	1
Study Duration	24 months
Individual Participation Duration	Approximately 14-17 months depending upon duration of deployment to malarious area.
Description of Agent or Intervention (Investigational Products)	<p><u>Sanaria® PfSPZ Vaccine</u>: Radiation attenuated, aseptic, purified, cryopreserved <i>Plasmodium falciparum</i> sporozoites (PfSPZ) administered by direct venous inoculation (DVI).</p> <p><u>Normal Saline (0.9% NaCl)</u>: The placebo control administered by DVI.</p> <p><u>Sanaria® PfSPZ-CVac</u>: Infectious, aseptic, purified, cryopreserved PfSPZ (Sanaria® PfSPZ Challenge) administered by DVI to individuals taking chloroquine (CQ) chemoprophylaxis.</p> <p><u>Normal Saline (0.9% NaCl)</u>: The placebo control administered by DVI to individuals taking CQ chemoprophylaxis.</p> <p>Diluent. The diluent for PfSPZ Vaccine and PfSPZ Challenge will be phosphate buffered saline (PBS) with 1% human serum albumin.</p>
Objectives	<p>Primary:</p> <ol style="list-style-type: none"> 1. To assess the safety and tolerability of PfSPZ Vaccine and PfSPZ-CVac compared to placebo in Indonesian soldiers. 2. To assess the protective efficacy (vaccine efficacy = VE) of PfSPZ Vaccine and PfSPZ-CVac against first clinical malaria cases caused by <i>P. falciparum</i> (Pf) identified by thick blood smear (TBS) microscopy or rapid diagnostic testing (RDT) in naturally exposed Indonesian soldiers. <p>Secondary:</p> <ol style="list-style-type: none"> 1. To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against first infections caused by Pf identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers. 2. To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against first clinical malaria cases caused by <i>P. vivax</i> (Pv) identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers. 3. To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against first infections caused by Pv identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers. 4. To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against relapsing infection from latent liver stages of Pv identified post-exposure in a malaria-free area. 5. To identify humoral immune responses that predict VE of PfSPZ Vaccine and/or PfSPZ-CVac.

	<p>Exploratory:</p> <ol style="list-style-type: none"> 1. To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against all cases of clinical malaria caused by Pf and Pv identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers. 2. To assess the VE of PfSPZ Vaccine and PfSPZ-CVac for reducing the number of asymptomatic Pf and Pv malaria infections. 3. To identify cellular immune responses that predict VE of PfSPZ Vaccine and PfSPZ-CVac. 4. To identify transcriptome or biomarker (protein) signatures that predict VE. 5. To identify markers of latent infection with Pv.
Design	<p>The study is a double-blind, randomized, placebo-controlled clinical trial that will assess the safety, tolerability, immunogenicity and vaccine efficacy (VE) of PfSPZ Vaccine and PfSPZ-CVac against naturally occurring malaria in Indonesian soldiers deployed to eastern Indonesia. Participants will receive three doses by DVI of: 1) PfSPZ Vaccine, on days 1, 8, and 29 (Group 1), 2) normal saline (NS), on days 1, 8, and 29 as a control for Group 1 (Group 2), 3) PfSPZ Challenge, on days 1, 29, and 57 with weekly CQ prophylaxis (Group 3), or 4) NS, on days 1, 29, and 57 as a control for Group 3, also with weekly CQ prophylaxis (Group 4). The PfSPZ Vaccine dose will be 9×10^5 PfSPZ. The PfSPZ Challenge dose will be 2×10^5 PfSPZ. CQ will administered as a loading dose of CQ (10mg/kg CQ base) by mouth 2 days prior to the first PfSPZ Challenge or NS administration, followed by 9 weekly doses of maintenance dose CQ (5 mg/kg CQ base) (10 administrations in total), in Groups 3 and 4.</p> <p>A total of 372 participants will be randomized 1.0:0.5:1.0:0.5 to the 4 groups (see below). Participants will be recruited from an Indonesian army battalion based in an area with no or minimal malaria transmission. All participants will be healthy males aged 18 to 55 years. They will be immunized at their home bases, and then deployed to eastern Indonesia for ~6-9 months where malaria is intensely transmitted. Standard safety, tolerability, and parasitology data will be collected before deployment during the immunization period.</p> <p>Study Arms:</p> <ol style="list-style-type: none"> 1. Group 1 (n=124): Three doses of 9×10^5 PfSPZ of PfSPZ Vaccine. 2. Group 2 (n=62): Three doses of NS. 3. Group 3 (n=124): Three doses of 2×10^5 PfSPZ of PfSPZ Challenge and weekly CQ. 4. Group 4 (n=62): Three doses of NS and weekly CQ. <p>After immunization is completed, there will be two follow up periods:</p> <p><u>Follow-up period #1.</u> This occurs 10 days from the time of arrival at the deployment site in eastern Indonesia to the end of deployment (~6-9 months) and for 10 additional days.</p> <p><u>Follow-up period #2.</u> This occurs for 24 weeks post deployment when the troops have returned to the malaria-free area of Indonesia.</p> <p>Follow-up during period #1 is designed to provide both active and passive surveillance for clinical malaria, the primary outcome variable. Active surveillance</p>

	<p>for clinical malaria will be done through interviews with each participant every two weeks by a clinical team member. The participant will be asked about malaria symptoms and body temperature will be measured. Any participant with symptoms or signs consistent with malaria will have blood sampled for malaria diagnostics. The primary diagnostic test will be thick blood smear (TBS) that will be read promptly except for soldiers in remote postings, in which case it will be a rapid diagnostic test (RDT) with a confirmatory blood smear made but read later. Passive surveillance will be done by encouraging soldiers with symptoms to report to the medical clinic at any time. Malaria confirmed by TBS or RDT will be immediately treated with supervised dihydroartemisinin-piperaquine.</p> <p>Active surveillance will also be done for malaria infection, the secondary outcome variable, by sampling blood for blood smear every four weeks in all participants, even if they are not experiencing signs or symptoms of malaria. However, blood smears from asymptomatic individuals will be read retrospectively to avoid interfering with surveillance for clinical malaria, the primary outcome variable. This is because immediate reading of blood smear from asymptomatic individuals could lead to identification and treatment of an infection that would otherwise have soon presented as a case of clinical malaria, leading to undercounting bias.</p> <p>Follow-up during period #2 will be the same with respect to passive case detection but may or may not include active surveillance. During follow-up period #2, unlike follow-up period #1, there will be radical cure with primaquine for participants having TBS confirmed infection by Pv.</p>
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Study schema for immunizations with PfSPZ Vaccine and PfSPZ-CVac.



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1 INTRODUCTION

1.1 Study Rationale

1.1.1 The need for a malaria vaccine

Malaria is a major global health problem, occurring throughout the tropics, subtropics, and in some temperate climates like the Korean peninsula. In 2019 the World Health Organization estimated that there were 229 million cases and 409,000 deaths [1], with some authorities providing higher estimates [2, 3]. Malaria is also a major contributor to poverty, restricting economic growth, stunting educational achievement, and perpetuating the poor access to public and clinical health services that often characterize malaria-endemic areas.

Like other tropical countries, Indonesia suffers heavily from malaria. Among Indonesia's 270 million people, over 150 million live at risk of malaria caused by any one of the five *Plasmodium* species known to cause this infection in humans – *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. The two dominant species in Indonesia are *P. falciparum* (Pf) and *P. vivax* (Pv), accounting for over 95% of infections and occurring at about equal frequencies [4]. Endemic transmission occurs on every major island, but it is relatively rare on the heavily populated and highly developed islands of Java, Bali and large areas of Sumatra. Beyond those islands, stable endemic malaria dominates the vast undeveloped rural areas of Indonesia, being especially severe in the relatively impoverished and isolated eastern half of Indonesia, such as the province of Papua.

The ideal intervention for malaria would be an effective vaccine. However, there are no licensed vaccines against malaria, or against any human parasitic disease. Until recently, the only candidate malaria vaccine with any promise has been GSK's RTS,S/AS01 vaccine (Mosquirix), a recombinant protein subunit vaccine targeting the surface coat of the Pf sporozoite, which is the infectious form of the parasite injected by the mosquito vector. In a Phase 3 study conducted in Africa, RTS,S/AS01 vaccine demonstrated efficacy in reducing the rate of *clinical* malaria due to Pf in infants and young children [5] but its effects on *severe malaria* and *mortality* were less clear. RTS,S has since been evaluated in a pilot program in 800,000 children in three African countries and has shown modest benefits in reducing hospitalizations for severe malaria but no detectable impact on mortality [6]. WHO has nonetheless recommended wider roll-out in sub-Saharan Africa countries for immunizing infants and young children as part of the EPI. The benefit of RTS,S/AS01 is limited to *clinical* cases in pediatric populations. Any effect on the *incidence of parasitemia* in the Phase 3 study is not yet public, and in the one earlier study where this key outcome was evaluated in adults [7] the effect on the incidence of parasitemia as measured by active surveillance using thick blood smears was not statistically significantly different from controls. In other words, RTS,S/AS01 appears to reduce clinical manifestations of the infection, but not the acquisition of the infection. Because vaccinees still develop parasitemia and transmit malaria, RTS,S/AS01 is not planned for use in malaria elimination programs. It is also not potent enough to be used for preventing malaria in travelers to malaria-endemic areas.

1.1.2 Whole sporozoite-based malaria vaccines

What is needed is a vaccine with an excellent safety profile that induces high level, long-term sterilizing immunity to malaria infection. Preventing infection would block transmission, and if administered to the majority of a population, such a vaccine could eliminate malaria from a

geographically defined area. Fortunately, there is a new class of vaccines under development that is more potent than RTS,S/AS01 and that is targeted for use to address this objective. Published and unpublished clinical trials of Sanaria® PfSPZ Vaccine and Sanaria® PfSPZ-CVac administered intravenously have demonstrated that durable protection against infection can be achieved in malaria-naïve and malaria-exposed adults, with studies of protection in pediatric populations underway. Both vaccine approaches use aseptic, purified, cryopreserved, metabolically active whole Pf sporozoites (SPZ) as the immunogen. The vaccines induce antibody responses targeting the SPZ stage, and cell-mediated immunity (CMI) targeting the liver stages, with the latter, CMI, thought to be the primary mechanism of protection. By blocking development in the liver, both vaccines are able to completely prevent blood stage infection. This protects the recipient from clinical disease and also eliminates ongoing transmission, which cannot occur without the development of gametocytes in the blood. The whole SPZ approach to malaria vaccination and the respective clinical development plans for PfSPZ Vaccine and PfSPZ-CVac have been reviewed [8].

PfSPZ Vaccine is composed of PfSPZ that have been attenuated by irradiation during manufacturing, making them safe to inject into humans. Although the PfSPZ are metabolically active and motile, and are able to invade hepatocytes, they are not able to multiply in hepatocytes due to radiation damage, and their development halts in the early hepatic stages. No parasitemia ever occurs, and the arrested organisms cause no signs or symptoms of illness. With PfSPZ-CVac, on the other hand, the PfSPZ are fully infectious, composed of the same PfSPZ as PfSPZ Vaccine (manufactured identically) but without the irradiation step. These are called Sanaria® PfSPZ Challenge, as they can be used to purposefully infect (challenge) humans. To attenuate the PfSPZ of PfSPZ Challenge, the vaccine recipient takes an oral antimalarial drug, called the “partner drug,” that kills the PfSPZ in vivo. PfSPZ-CVac is the name given to the combination of PfSPZ Challenge + partner drug (“CVac” meaning “chemoprophylaxis vaccination”). The gold-standard partner drug, chloroquine (CQ), is a selective blood schizonticide that does not kill the parasites until they break out of the liver into the blood. This means that the parasites multiply up to 50,000-60,000 fold in the liver, thereby increasing the immunogen load as well as the diversity of antigens expressed. This induces a more potent immune response than the irradiated PfSPZ of PfSPZ Vaccine, which arrest early in liver stage development and do not multiply. PfSPZ-CVac therefore has the advantage of inducing protection with much lower doses of PfSPZ, but has the disadvantage that the recipient experiences a brief period of parasitemia before the parasites are killed by the CQ, usually on +7, +8 and +9 days after injection of PfSPZ Challenge. At this point, it is not clear which of these two vaccine approaches will prove to be the most protective, adequately safe and the most likely to achieve malaria elimination.

Recent data from trials in the USA, EU and Africa show that high level protection can be achieved with both vaccine approaches. Vaccine efficacy (VE) has been assessed using controlled human malaria infection (CHMI), where vaccine recipients and controls are purposefully exposed to infectious mosquito bites or injected PfSPZ, and by field studies, where vaccine recipients and controls are exposed to naturally transmitted malaria in endemic settings. The following have all been demonstrated in studies of PfSPZ Vaccine in the USA: 100% protection against homologous malaria strains using CHMI at 3 weeks after immunization (where “homologous” means the strain of malaria used in the challenge is the same strain as used in the vaccine), 79-80% protection against heterologous CHMI at 3 weeks after immunization (where “heterologous” means the strain of malaria used in the challenge is different from the strain of malaria used in the vaccine) including following

immunization with the same PfSPZ Vaccine regimen as proposed for the Indonesia trial, and long term (14 month) protection against homologous CHMI [9-12]. PfSPZ-CVac has likewise demonstrated high level protection in a study in Germany, achieving 100% protection against homologous CHMI at 10 weeks after immunization [13], and more recently has broken all prior malaria vaccine efficacy records by demonstrating 100% protection against heterologous CHMI at 12 weeks following immunization with the same PfSPZ-CVac regimen as proposed for the Indonesia trial [14]. CHMI studies in adults previously exposed to malaria have shown similar findings. In a study in Tanzania, 6/6 (100%) adult participants undergoing CHMI 3 to 10 weeks after receiving 3 doses of 9.0×10^5 PfSPZ of PfSPZ Vaccine administered at 8-week intervals were protected [15] and in Mali, 30/30 (100%) adults receiving 3 doses of 1.8×10^6 PfSPZ of PfSPZ Vaccine also administered at 8 week intervals were protected (Sissoko and Healy, unpublished). When tested for efficacy in the field, PfSPZ Vaccine prevented naturally transmitted Pf *infection* over the six month transmission season in three studies in Malian adults, achieving 51%, 52% and 42% VE by time-to-event analysis, and 29%, 24% and 26% VE by proportional analysis [16, 17]. Similar data were obtained in a study in Burkina Faso, which showed VE of 48% and 38% against Pf *infection* by the two efficacy approaches over a full malaria transmission season (Sirima and Laurens, unpublished). In both the Burkina study and the most recent Mali study, ongoing protection was documented during a second transmission season without the need for a booster dose. Protection against *clinical malaria* was also measured in the most recent Mali study, which was noteworthy because the same immunization regimen was used in that study as proposed for the current trial in Indonesia. This was 46% and 51% VE against *clinical malaria* in the first and second transmission seasons by time-to-event analysis and 33% and 37% by proportional analysis. In other words, VE was a bit higher against *clinical malaria* than against *infection*, and showed no loss from season to season. These promising results support the continuing development of whole PfSPZ malaria vaccines. Sanaria's objective is to use PfSPZ-based products in mass vaccination programs (MVPs) aimed at regional elimination of malaria. Whole PfSPZ vaccines are also being developed to protect malaria-naïve travelers staying for limited periods in malaria endemic areas, for children living in regions of Africa with well-defined transmission seasons (such as the Sahel) and for pregnant women.

The research proposed in this protocol is a critical part of the whole PfSPZ-based vaccine development program. It will constitute the first assessment of these two vaccine products for the prevention of naturally transmitted Pf malaria in the field in the same clinical trial. It will also mark the first time that either vaccine has been tested in Asia. Both vaccines will be administered intravenously (IV) using an easy, rapid, nearly pain-free technique called direct venous inoculation (DVI). Three doses of each will be administered at 0, 4 and 8 weeks (PfSPZ Challenge) or 0, 7 and 28 days (PfSPZ Vaccine). The partner drug for PfSPZ Challenge will be CQ. The proposed clinical trial will be performed in the context of a military deployment of malaria-naïve (or minimally malaria-exposed) soldiers from the Indonesian Army. A battalion will be selected that is based in a relatively malaria-free area of Indonesia, most likely on the islands of Java, Sumatra or Bali. The soldiers will be immunized at their home base with PfSPZ Vaccine, PfSPZ-CVac, or normal saline placebo using a randomized, double-blind, placebo-controlled design. The battalion will then be deployed to an area in eastern Indonesia such as Papua where malaria transmission is intense. According to Indonesian military medical doctrine, the soldiers will not be taking antimalarial chemoprophylaxis. This will enable the accurate measurement of malaria incidence in vaccinees and controls. If one or both vaccines demonstrate high level efficacy, such a vaccine could meet many of the public health needs associated with the scourge

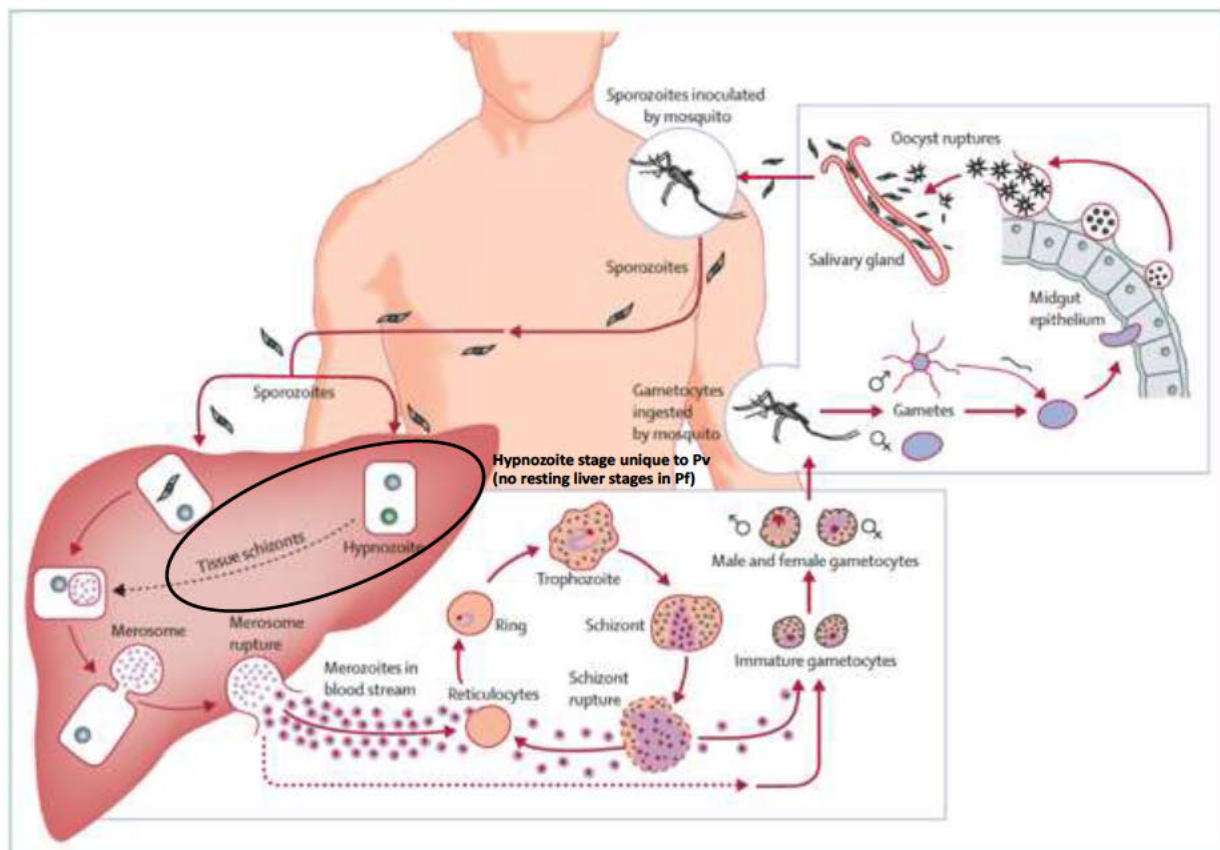
of malaria, and greatly mitigate the widespread misery and death caused by malaria among some of the most vulnerable and invisible people on Earth.

1.1.3 Malaria parasite life cycle and cross-species protection

As described above, Pf and Pv malaria are responsible for the vast majority of malaria-induced disease and death in Indonesia. The general malaria life cycle is complex and is depicted in Figure 1. Female Anopheles mosquitoes inject the worm-like SPZ which travel to the liver and invade hepatocytes. About six to ten days later, each infected hepatocyte releases thousands of merozoites into the bloodstream. Each merozoite can invade a different red blood cell. Within the red blood cells, merozoites multiply asexually over the course of 48 hours to produce 15-30 new merozoites per dividing stage of the parasite, also called the schizont. At 48 hours after merozoite invasion, each schizont-infected red blood cell bursts and releases this new generation of merozoites, each able to invade other red blood cells. The number of parasites grows logarithmically, leading to the clinical signs and symptoms and malaria. In addition to the asexual merozoites, sexual forms called gametocytes develop in the blood, and these transmit the parasite back to the mosquito vector when the mosquito takes a blood meal, completing the transmission cycle.

The principal difference between the life cycles of Pf and Pv is the presence of dormant liver stage parasites called hypnozoites in Pv that do not occur in Pf (see black oval in **Figure 1**). These parasites awaken in the weeks and months following infection to cause a renewed clinical attack, or relapse. This difference is an important reason for the persistence of Pv and the difficulty of breaking the transmission cycle of this parasite. Drugs that treat clinical malaria do not kill the hypnozoites, and a special class of drugs (8-amino quinolines such as primaquine) is needed. The concern about Pv hypnozoites and relapsing Pv infection adds an interesting component to the proposed clinical trial. During the initial deployment to Papua, the trial will measure the protection afforded by PfSPZ Vaccine and PfSPZ-CVac against not just *primary Pf* but also *primary Pv* infections (with clinical malaria being the primary outcome variable and infection being secondary). Then, during a six-month period following re-deployment to the malaria-free home base, the trial will measure the protection afforded against *relapsing Pv* infection.

Figure 1. *Plasmodium falciparum* and *Plasmodium vivax* life cycle



Both PfSPZ Vaccine and PfSPZ Challenge are manufactured from a single strain of *Pf* malaria, the NF54 strain believed to be of African origin. Will the immune response induced by this *Pf* strain be active against *Pv*? There is a strong rationale for anticipating that there will be cross-protection between these two most important malaria species. Protection by PfSPZ is thought to be mediated primarily by CD8⁺ T cells that recognize parasite-infected hepatocytes [9, 18-21]. Since the *Pf* [22] and *Pv* [23] proteomes share hundreds of thousands of identical 8-11 amino acid sequences, each of which could be a shared CD8⁺ T cell epitope, there could be cross protection. Furthermore, when irradiated *P. yoelii* (Py) or *P. berghei* (Pb) SPZ were used to immunize mice, mice were protected against challenge with either species, demonstrating cross-protection [24] (**Table 1** below). On a genome and proteome basis, *Pf* and *Pv* are no more distant from each other than are Py and Pb. Since Indonesian military personnel generally become infected with *Pf* and *Pv* at equal rates, this trial offers a unique opportunity to definitively assess two key questions: (1) Can the immune responses induced by the *Pf* vaccines attack and kill the primary hepatic stages of *Pv* (as measured by a reduction in first *Pv* event attacks in soldiers while actively exposed to infectious *Anopheles* mosquitoes in the field)? (2) Can the same immune responses attack and kill the latent *Pv* hypnozoites resting in the liver (as measured by a reduction in first *Pv* relapses observed following cessation of exposure to infection)?

Table 1. Left: Cross-protection between PySPZ and PbSPZ in BALB/c mice. Right: Protective immunity in BALB/c mice immunized with radiation-attenuated PySPZ with and without CD8⁺ T cell depletion [24].

Immunization	Challenge SPZs ^a	Number protected/ number challenged		Total (% protected)
		Experiment 1	Experiment 2	
Irradiated PySPZ	<i>P. yoelii</i>	6/6	7/7	13/13 (100)
Irradiated PySPZ	<i>P. berghei</i>	4/6	3/7	7/13 (54)
Irradiated PbSPZ	<i>P. berghei</i>	6/6	7/7	13/13 (100)
Irradiated PbSPZ	<i>P. yoelii</i>	5/6	7/7	12/13 (92)
Naive	<i>P. yoelii</i>	0/6	0/7	0/13 (0)
Naive	<i>P. berghei</i>	0/6	0/7	0/13 (0)

Immunization	T cell depletion	Challenge SPZs ^a	Number protected/ number challenged (% protected)
Irradiated PySPZ	Control	<i>P. yoelii</i>	6/6 (100)
Irradiated PySPZ	CD8 ⁺	<i>P. yoelii</i>	0/6 (0)
Irradiated PySPZ	Control	<i>P. berghei</i>	5/6 (83)
Irradiated PySPZ	CD8 ⁺	<i>P. berghei</i>	0/7 (0)
Naive	–	<i>P. yoelii</i>	0/6 (0)
Naive	–	<i>P. berghei</i>	0/6 (0)

1.2 Background

Sanaria Inc. was founded in 2002 to develop a vaccine based on the promising results from mosquito-administered PfSPZ [25, 26]. In these now classic studies dating back to the early 1970s, infected mosquitoes were irradiated, and then allowed to bite malaria-naïve humans who subsequently underwent CHMI. It was shown that > 90% of study participants were protected against homologous CHMI when first conducted within 10 weeks if the total number of infectious bites was 1000 or greater, and the majority of protected individuals were still protected when re-challenged up to 10 months after immunization [27]. The founding of Sanaria was based on the proposition that a vaccine composed of aseptic, purified, cryopreserved PfSPZ could be produced in compliance with current Good Manufacturing Practices (cGMPs) and should also be > 90% protective when injected parenterally [28]. After several years of research and development, highly purified, aseptic PfSPZ suitable for parenteral injection were manufactured successfully at Sanaria's Clinical Manufacturing Facility in Rockville, Maryland, USA [29]. Whether administered to humans as radiation-attenuated SPZ (PfSPZ Vaccine) or as non-attenuated SPZ (PfSPZ Challenge), the PfSPZ have been safe and well tolerated, with the PfSPZ themselves inducing almost no measurable side effects (see below). Thousands of vials of PfSPZ Vaccine, PfSPZ Challenge, or a third product PfSPZ-GA1 (genetically attenuated PfSPZ) that meet regulatory standards of purity, sterility, safety, and potency have now been manufactured in compliance with cGMPs [29]. Because the PfSPZ are eukaryotic cells, they must be stabilized at very cold temperatures, the same requirement as for human eggs and sperm. This is done using liquid nitrogen vapor phase (LNVP). Vials of cryopreserved PfSPZ are removed from the LNVP, thawed, diluted with phosphate buffered saline (PBS) and human serum albumen (HSA), and injected by the DVI route. By conducting an extensive stability program using PfSPZ stored in LNVP, it has been shown that the PfSPZ are extremely stable over the four years of testing mandated for each lot (Sim, unpublished data). It has also been shown that the cost and logistics of distributing cryopreserved whole sporozoite vaccines in the field are high favorable, due to the fact that electricity is not needed to maintain the cold chain [30].

1.2.1 Preclinical investigations of PfSPZ Vaccine and PfSPZ Challenge (PfSPZ-CVac)

1.2.1.1 Toxicity studies of PfSPZ Vaccine

The safety and immunogenicity of PfSPZ Vaccine administered by subcutaneous (SC), intradermal (ID), and IV routes was demonstrated in New Zealand White (NZW) rabbits and mice. Three preclinical toxicology studies in rabbits, giving 5-7 doses of 1.35×10^5 PfSPZ by the ID, SC and IV routes were performed with the radiation-attenuated PfSPZ Vaccine. In all 3 studies, PfSPZ Vaccine was safe and well tolerated. The vaccine was also safe and well tolerated in the mouse study. More details are available in the PfSPZ Vaccine Investigator's Brochure (IB). No toxicity studies were done for PfSPZ Challenge.

1.2.1.2 Biodistribution studies of PfSPZ Vaccine

Formal biodistribution studies were performed, which examined the distribution and persistence in mouse tissues of Pf DNA following SC, ID, or IV inoculation of a single 1.35×10^5 PfSPZ dose of PfSPZ Vaccine. The studies showed that PfSPZ Vaccine was safe and well tolerated in mice and that there was no persistence of PfSPZ in the host tissue beyond 144 hours. The dose of sporozoites in these studies was equivalent to the highest dose administered in the first two Phase 1 clinical trials of PfSPZ Vaccine, a dose that has now been shown to be safe and well tolerated in adult and pediatric participants administered PfSPZ Vaccine by SC, ID, and/or IV routes. More details are available in the Investigator's Brochure (IB).

1.2.1.3 No toxicity or biodistribution studies required for PfSPZ-CVac

Based on the results of pre-clinical and clinical studies of Sanaria PfSPZ Vaccine, clinical studies of PfSPZ Challenge were initiated without additional pre-clinical toxicology or biodistribution studies.

1.2.2 Clinical investigations of PfSPZ Vaccine and PfSPZ Challenge (PfSPZ-CVac)

1.2.2.1 PfSPZ Vaccine

There have been 20 clinical trials of PfSPZ Vaccine conducted at 4 sites in the USA, in 2 countries in Europe and in 6 countries in Africa, and a 21st trial is now underway in Seattle, WA, USA. As of October 1, 2021, 5606 doses of PfSPZ Vaccine (over 5.2 billion PfSPZ) have been administered to 1726 participants aged 5 months to 61 years; this includes 873 doses administered by DVI to 330 infants. The PfSPZ have been safe and well tolerated. Highlights of these studies are presented here. Additional details are included in the PfSPZ Vaccine Investigator's Brochure (IB), which is updated on an annual basis.

1. *First clinical trial of PfSPZ Vaccine*: PfSPZ Vaccine was administered ID or SC in a first-in-humans Phase 1 clinical trial in 80 healthy, malaria-naïve adults at the Naval Medical Research Center and the University of Maryland [21]. PfSPZ Vaccine was safe, fully attenuated by irradiation (no breakthrough blood stage infections) and well tolerated in human participants. Furthermore, the vaccine induced antibody and T-cell immune responses, and protected several participants against controlled human malaria infection (CHMI). However, when administered into or under the skin, PfSPZ Vaccine did not confer adequate protection. Studies of PfSPZ Vaccine in non-human primates demonstrated that the SC route of administration was suboptimal, whereas IV administration greatly increased PfSPZ-

specific immune responses, especially the frequency of liver resident CD8+ T cells recognizing PfSPZ [21]. This finding justified further clinical testing of PfSPZ Vaccine using the intravenous (IV) route.

2. 100% protection achieved after IV administration: Beginning in October 2011, a study of the safety, tolerability, immunogenicity and vaccine efficacy (VE) of PfSPZ Vaccine administered IV was initiated at the Vaccine Research Center (VRC), National Institutes of Allergy and Infectious Diseases (NIAID), NIH, Bethesda [9]. A catheter was placed in the peripheral vein of the test participants, and the vaccine administered as an IV push through the catheter, resulting in much improved immunogenicity. This trial, called VRC 312, showed that PfSPZ Vaccine was safe and well tolerated as the doses were escalated from 2×10^3 to 7.5×10^3 to 3×10^4 to 1.35×10^5 PfSPZ/dose, with concurrence of the Safety Monitoring Committee (SMC) and FDA at each dose escalation. There were no severe adverse events (AEs) considered related to vaccination and no allergic reactions. A vaccine dose response with regard to antibody and T cell responses was observed. CHMI in the higher dose groups (4 or 5 doses of 1.35×10^5 PfSPZ, total dosage of 5.4 – 6.75×10^5 PfSPZ) was performed 3 weeks after the last immunization using homologous parasites. VE was 67% (6/9) in participants who received 4 doses and 100% (6/6) in participants who received 5 doses [9].

3. VE extends to heterologous parasite strains, lasts >1 year, can be achieved after 3 doses, and can be achieved using condensed regimens: The publication of the landmark VRC312 study in *Science* in 2013 describing 100% protection against CHMI led to an enthusiastic international response, and multiple research groups have joined the International PfSPZ Consortium (I-PfSPZ-C), partnering with Sanaria to conduct 19 additional Phase 1 or Phase 2 trials of PfSPZ Vaccine (**Table 2**). The first of these trials (VRC 314) also used the IV catheter route, but all subsequent studies have used the simpler, more rapid direct venous inoculation (DVI) route, where the vein is punctured directly and nearly painlessly with a 25-gauge needle and the vaccine in 0.5 mL of diluent is injected over a few seconds, a procedure that in most recipients is extremely well tolerated and causes no AEs. DVI is now the standard for administration, although smaller volumes of diluent are also being assessed. Five of the 18 newer (DVI) trials have been performed (or are ongoing) in malaria-naïve adults (studies # 6, 11, 13 and 20, plus the trial listed in the footnote to **Table 2**) and 13 have been performed in malaria-exposed Africans including adults, teenagers, children and infants (studies # 4, 5, 7-9, 10, 12 and 14-19 in **Table 2**).

Table 2. Chronological Listing of Trials of PfSPZ Vaccine

Study Identifier (Clinicaltrials.gov) Start Date	Study Design Summary	PfSPZ Vaccination Schedules Evaluated (Dose, Route, Number of administrations)	Vaccine groups and Numbers of Participants
1. NMRC/UMB CVD (NCT01001650) May 2009 (completed)	Phase 1, open-label, dose- escalation with CHMI in USA (ID, SC only)	7.5×10^3 SC x 4; 7.5×10^3 ID x 4; 3×10^4 SC x 4; 3×10^4 ID x 4; 1.35×10^5 SC x 4 or 6; 1.35×10^5 ID x 4 or 6	Malaria-naïve adults: 80
2. VRC 312 (NCT01441167) Oct 2011 (completed)	Phase 1, open-label, dose- escalation with CHMI in USA (IV by catheter)	2×10^3 IV x 2; 7.5×10^3 IV x 4 or 6; 3×10^5 IV x 4 or 6; 1.35×10^5 IV x 4 or 5	Malaria-naïve adults: 40

Study Identifier (Clinicaltrials.gov) Start Date	Study Design Summary	PfSPZ Vaccination Schedules Evaluated (Dose, Route, Number of administrations)	Vaccine groups and Numbers of Participants
3. VRC 314 (NCT02015091) Dec 2013 (completed)	Phase 1, open-label, dose- escalation, regimen comparison with CHMI in <u>USA</u> (IV by catheter or IM)	2.2x10 ⁶ IM x 4; 1.35x10 ⁵ IV x 4 + 4.5x10 ⁵ IV boost; 2.7x10 ⁵ IV x 3 or 4; 2.7x10 ⁵ IV x 2 + 4.5x10 ⁵ IV x 2; 9.0x10 ⁵ IV x 3	Malaria-naïve adults: 93
Administration in all following trials by DVI only			
4. Mali 1 (NCT01988636) Jan 2014 (completed)	Phase 1, randomized, double-blind placebo- controlled* field efficacy in <u>Mali</u>	1.35x10 ⁵ + 2.7x10 ⁵ ; 2.7x10 ⁵ x 5	Malaria-exposed adults: 58
5. BSPZV1 (NCT02132299) May 2014 (completed)	Phase 1, randomized, double- blind placebo- controlled* with CHMI (by needle and syringe) in <u>Tanzania</u>	3x10 ⁴ ; then 1.35x10 ⁵ ; then 2.7x10 ⁵ 1.35x10 ⁵ x 5; 2.7x10 ⁵ x 5	Malaria-exposed adults: 49
6. WRAIR 2080 (NCT02215707) Jun 2014 (completed)	Phase 1, open-label, regimen comparison with CHMI in <u>USA</u>	2.7x10 ⁵ x 5; 4.5x10 ⁵ x 3	Malaria-naïve adults: 45
7. EGSPZV1 (NCT02418962) Mar 2015 (completed)	Phase 1, open-label, dose- escalation in <u>Equatorial Guinea</u>	1.35x10 ⁵ ; then 2.7x10 ⁵ 2.7x10 ⁵ x 3	Malaria-exposed adults: 23
8. BSPZV2 (NCT02613520) Dec 2015 (completed)	Phase 1 dose escalation, double-blind, randomized, placebo-controlled* with CHMI (by needle and syringe) in <u>Tanzania</u>	Adults, older children: 9x10 ⁵ x 3; then 1.8x10 ⁶ x 3 Younger children, infants: 4.5 x 10 ⁵ x 3; then 9x10 ⁶ x 3	Malaria-exposed adults: 12 children: 36 infants: 15
9. Mali 2 (NCT02627456) Jan 2016 (completed)	Phase 1 dose escalation with CHMI followed by Phase 2 randomized, double-blind, placebo- controlled* field efficacy in <u>Mali</u>	Ph 1: 4.5x10 ⁵ x 1; then 9x10 ⁵ x 1; then 1.8x10 ⁶ x 3 Ph 2: 1.8x10 ⁶ x 3	Malaria-exposed adults: 100
10. Burkina Faso 1 (NCT02663700) Apr 2016 (completed)	Phase 1 dose escalation followed by Phase 2, randomized, double-blind placebo-controlled* field efficacy in <u>Burkina Faso</u>	Ph 1: 4.5x10 ⁵ x 2; then 9x10 ⁵ x 2; then 1.8x10 ⁶ x 2; then 2.7x10 ⁶ x 2 Ph 2: 2.7x10 ⁶ x 3	Malaria-exposed adults: 32

Study Identifier (Clinicaltrials.gov) Start Date	Study Design Summary	PfSPZ Vaccination Schedules Evaluated (Dose, Route, Number of administrations)	Vaccine groups and Numbers of Participants
11. Warfighter 2 (NCT02601716) Apr 2016 (completed)	Phase 2, open-label, regimen comparison with CHMI in <u>USA</u>	4.5x10 ⁵ x 5 (Days 1, 3, 5, 7 and week 16); or 9x10 ⁵ x 3 (Weeks 1, 9, 17); or 1.8x10 ⁶ x 3 (Weeks 1, 9, 17); or 2.7x10 ⁶ x 1 + 9x10 ⁵ x 2 (Weeks 1, 9, 17)	Malaria-naïve adults: 60
12. KSZPV1 (NCT02687373) Jul 2016 (completed)	Phase 1 dose escalation followed by Phase 2 double- blind, randomized, placebo- controlled* with field efficacy in <u>Kenya</u>	Ph 1 - Older children: 4.5x10 ⁵ x 1; then 9x10 ⁵ x 2; then 1.8x10 ⁶ x 2 Ph 1 - Younger children, infants: 1.35x10 ⁵ x 1; then 2.7x10 ⁵ x 1; then 4.5x10 ⁵ x 1; then 9x10 ⁵ x 2; then 1.8x10 ⁶ x 2, all Ph 2 - Infants: 4.5x10 ⁵ , 9x10 ⁵ , or 1.8x10 ⁶ , all x 3	Malaria-exposed children: 100 infants: 401
13. MAVACHE (NCT02704533) Sep 2016 (completed)	Phase 1 dose escalation, regimen-condensation and dose number reduction with CHMI in <u>Germany</u>	9x10 ⁵ x 3 (Days 1, 8, 29); then 1.8x10 ⁶ x 2 (Days 1, 8); then 2.7x10 ⁶ x 2 (Days 1, 8)	Malaria-naïve adults: 42
14. EGSPZV2 (NCT02859350) Nov 2016 (completed)	Phase 1 dose escalation, randomized double-blind, placebo-controlled* with head-to-head PfSPZ Vaccine and PfSPZ-CVac comparison in <u>Equatorial Guinea</u>	Adults (PfSPZ Vaccine): 2.7x10 ⁶ x 3 Adults (PfSPZ-CVac): 1x10 ⁵ x 3 Children, infants (PfSPZ Vaccine): 1.8x10 ⁶ x 3	Malaria-exposed adults: 52** children: 36 infants: 15
15. BSPZV3a (NCT03420053) Feb 2018 (completed)	Phase 1 dose escalation, randomized double-blind, placebo-controlled* with CHMI in <u>Tanzania</u>	4.5x10 ⁵ x 5 (Days 1, 3, 5, 7 and 29); or 9x10 ⁵ x 5 (Days 1, 3, 5, 7 and 29)	Malaria-exposed HIV- and HIV+ adults: 21
16. MSPZV3 (NCT03510481) June 2018 (completed)	Phase 2 double-blind, randomized, placebo- controlled* with field efficacy in <u>Mali</u>	9x10 ⁵ x 3 (Days 1, 8 and 29); or 9x10 ⁵ x 3 (Weeks 1, 9, 17)	Malaria-exposed adults: 210
17. LaSPZV1 (NCT03521973) June 2018 (completed)	Phase 2 double-blind, randomized, placebo- controlled* with field efficacy in <u>Gabon</u>	9x10 ⁵ x 3 (Days 1, 8 and 29)	Malaria-exposed children: 200
18. EGSPZV3 (NCT03590340) Aug 2018 (completed)	Phase 1 double-blind, randomized, placebo- controlled* with CHMI in <u>Equatorial Guinea</u>	9x10 ⁵ x 3 (Days 1, 8 and 29); or 9x10 ⁵ x 5 (Days 1, 3, 5, 7 and 29); or 9x10 ⁵ x 5 (Days 1, 3, 5, 7 and Week 17); or 9x10 ⁵ x 4 (Days 1, 3, 5, 7)	Malaria-exposed adults: 104

Study Identifier (Clinicaltrials.gov) Start Date	Study Design Summary	PfSPZ Vaccination Schedules Evaluated (Dose, Route, Number of administrations)	Vaccine groups and Numbers of Participants
19. MSPZV4 (NCT03510481) June 2019 (completed)	Phase 2 double-blind, randomized, placebo- controlled* with field efficacy in Mali	9x10 ⁵ x 3 (Days 1, 8 and 29); or 1.8x10 ⁶ x 3 (Days 1, 8 and 29)	Malaria-exposed women of child-bearing potential: 300
20. Warfighter 3 (NCT04966871) October 2021 (ongoing)	Double-blind, placebo- controlled* with heterologous CHMI at 4, 6 and 10 weeks after immunization	9x10 ⁵ x 3 (Days 1, 8, 29)	Malaria-naïve adults: 54
*The placebo control used in all trials is normal saline; ** 20/52 adult participants in EGSPZV2 received PfSPZ-CVac.			
In these trials, dosing schedules have evaluated various intervals between vaccinations ranging from 4 to 16 weeks.			
This table omits one trial of a genetically attenuated sporozoite vaccine (PfSPZ-GA1) conducted in the Netherlands that used PfSPZ Vaccine as a comparator (total number of trials is therefore 21).			

The four completed IV/DVI trials in malaria-naïve participants (studies #3, 6, 11 and 13 **Table 2**) demonstrated several important findings:

A. *The degree of protection depended on the dose of PfSPZ administered:* There has been a clear dose response seen up to doses of about 9.0x10⁵ PfSPZ per injection. For example, VE against homologous CHMI conducted 3 weeks after a 3-dose regimen rose from 20% to 87% as the dose of PfSPZ administered was increased from 2.7x10⁵ to 4.5x10⁵ PfSPZ [10, 11]. As individual doses were increased above about 9.0x10⁵ PfSPZ, further improvements were not always seen. For example, in the Warfighter 2 trial, doubling 3-dose regimens from 9.0x10⁵ PfSPZ per dose to 1.8x10⁶ PfSPZ per dose did not improve VE against heterologous CHMI conducted at 3 or 6 months, and in the MAVACHE study, a trial underway in Tübingen, Germany, there were similar findings: doubling 2-dose regimens from 1.35x10⁶ PfSPZ to 2.7x10⁶ PfSPZ per dose did not improve protection against homologous CHMI at 3 weeks (Mordmüller, submitted).

B. *The number of doses affected protection:* This was illustrated by the MAVACHE trial, where two attempts at immunizing with 2 doses were unsuccessful. The difference between 3 and 5 doses is less marked. For example, 3 doses of 4.5x10⁵ PfSPZ gave slightly less protection (87%) than 5 doses of 2.7x10⁵ PfSPZ (92%) [11] even though both regimens used the same total dose of 13.5x10⁵ PfSPZ. Because this difference was small, 3 dose regimens became the standard, due to the improved logistics of 3 doses compared to 5.

C. *The protection induced by PfSPZ Vaccine extended to heterologous parasites:* In the field, most of the parasites transmitted by mosquitoes are heterologous, differing antigenically from the vaccine strain; therefore, demonstrating heterologous protection by CHMI is important. In the WRAIR 2080 trial, a 5-dose regimen similar to that providing 100% protection in VRC 312 protected 4/5 (80%) participants against heterologous CHMI at 3 weeks; the CHMI used mosquito-bite inoculation of Pf7G8, a clone of a Brazilian Pf strain that is antigenically divergent from PfNF54, indicating that potent cross-strain immunity is induced [11].

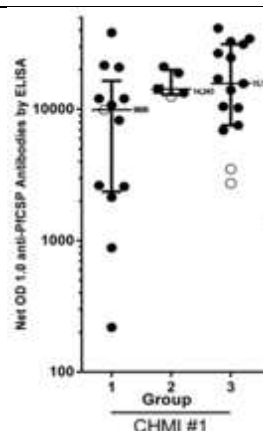
D. PfSPZ Vaccine gave protection lasting > 1 year: In the VRC 314 trial (conducted at VRC and UMB), 5/5 (100%) participants protected against homologous CHMI at ~20 weeks after immunization were sterilely protected against repeat homologous CHMI at 59 weeks [10] and 5/6 (83%) participants protected against homologous CHMI at 18 weeks were sterilely protected against heterologous (7G8) CHMI at 33 weeks [12]. Both homologous and heterologous protection were therefore durable for at least 8 months, likely for > 12 months.

E. Condensed regimens were highly protective: A Day 1, 3, 5, 7 (highly condensed) prime with a 16-week boost regimen in Warfighter 2 gave the best protection results yet seen in the development of PfSPZ Vaccine – 40% sterile protection against a stringent mosquito bite Pf7G8 CHMI conducted at 13 weeks after immunization. This was twice as high as the comparator 3-dose regimens administered over the standard 0, 8 and 16 weeks, despite a lower total dose of PfSPZ [31]. MAVACHE also showed encouraging results after a condensed regimen: 78.8% (14/17) participants were protected against 3-week or 10 week homologous CHMI following 3 doses of 9.0×10^5 PfSPZ at Day 1, 8 and 29, and 83.3% (10/12) against heterologous CHMIs at the same timepoints (Mordmüller, submitted).

F. Antibody levels to Pf circumsporozoite protein (CSP) measured by ELISA have correlated with protection against CHMI: An additional finding from VRC314 and WRAIR 2080 was that antibody levels to the main surface protein of PfSPZ, the Pf circumsporozoite protein (PfCSP), as measured by ELISA, correlated with protection in malaria-naïve individuals immunized with PfSPZ Vaccine (**Figure 2**) [10, 11]. Other humoral assays, such as anti-sporozoite immunofluorescence assays (IFA) and inhibition of SPZ invasion assays (ISI) have also shown associations with protection. The availability of these standardized assays and their association with protection suggest that it may be possible to develop one of these assays as a standardized measure of “vaccine take,” a surrogate marker for the cellular immune mechanisms thought to underlie protection induced by whole PfSPZ-based vaccines. Such an assay would strongly aid the development and use of PfSPZ-based vaccines.

Figure 2. Antibody responses in WRAIR 2080

participants. Protected participants are shown as filled (black) circles and unprotected participants as unfilled circles. For each of the three immunization groups, the interquartile ranges and the median values of responses of participants are shown. Assessment of antibodies was performed in sera from participants before immunization and 2 weeks after the last dose of PfSPZ Vaccine, which was ~1 week before CHMI. Antibodies to PfSPZ by IFA are reported as the reciprocal serum dilution at which the fluorescent units were 2×10^5 (AFU 2×10^5).



4. PfSPZ Vaccine is also safe, well tolerated and protective in malaria-exposed African adults, and safe and well tolerated in African children and infants: The 13 trials in malaria-exposed Africans

including adults, teenagers, children and infants (studies # 4, 5, 7-10, 12, and 14-19 in **Table 2**) have also demonstrated several important findings:

A. PfSPZ Vaccine induces durable protection against naturally transmitted Pf infection in Africa:

The first, randomized, double-blind, placebo-controlled trial testing efficacy against naturally transmitted malaria was conducted in Mali (#4 in **Table 2**). It showed 52% VE against Pf *infection* as measured by microscopic examination of thick blood smears during a 24-week follow-up period by time-to-event analysis, and 29% VE by proportional analysis [16]. At that time, this VE was the best protection ever achieved against naturally transmitted Pf infection in African adults (or the world). The attack rate in the placebo group was 93.2%, indicating intense transmission, yet PfSPZ Vaccine durably prevented malaria in a proportion of study participants. An interesting finding in the Mali trial and also other trials in African adults was that the antibody titers induced by PfSPZ Vaccine were lower than when the same dose was administered to malaria-naïve adults in the U.S. This may reflect the Pf malaria antigen-specific immune suppression caused by a lifetime of exposure to Pf malaria. Nevertheless, a correlation with PfCSP ELISA antibody titers was shown in this trial, indicating that this potential surrogate marker for protection may not be restricted to malaria-naïve adults protected against CHMI. It could also be that while antibody responses were lower in malaria-exposed adults, the cellular responses believed to be the basis of protection may not have been affected by prior exposure. The subsequent year, a similar trial was performed at a different site in Mali, using 3 doses of 1.8×10^6 PfSPZ at 0, +8 and +16 weeks rather than 5 doses of 2.7×10^5 PfSPZ at 0, +4, +8, +12 and +20 weeks, with incident Pf *infection* again measured over six months as before, and showed similar results, 51% VE by time-to-event and 24% VE by proportional analysis, demonstrating that a 3-dose regimen can be as effective as a 5-dose regimen (#9 in **Table 2**) [17]. A third field efficacy study has since been completed in malaria-exposed adults in Burkina Faso (#10 in **Table 2**); this trial tested 3 doses of 2.7×10^6 PfSPZ (total dose 8.1×10^6 PfSPZ) and showed 48% VE by time-to-event and 38% VE by proportional analysis; importantly, protection extended through a second malaria transmission season (Siriman and Laurens, unpublished). Finally, another, more recent trial in Mali, conducted in women of child-bearing potential (#19 in **Table 2**) in order to study the safety of PfSPZ Vaccine when administered prior to pregnancy, also showed significant protection against Pf *infection* that extended over two seasons: 42% and 62% VE in season 1 and 2, respectively, by time-to-event analysis using the modified intention-to-treat (m-ITT) population, and 26% and 45% VE by proportional analysis (also m-ITT). Of note, this trial used the same regimen as planned for the Indonesia trial. Based on FDA request, VE against *clinical malaria* was also measured, and also showed protection over two seasons: this was 49% and 56% VE in the first and second transmission seasons, by time-to-event analysis, and 36% and 42% by proportional analysis (m-ITT), indicating sustained protection.

B. PfSPZ Vaccine twice achieved 100% protection against short term homologous CHMI in Africa, and once on repeat CHMI 21 weeks later: CHMI has now been employed 54 times in 6 African countries to induce malaria infection or to assess PfSPZ Vaccine efficacy in African adults, using PfSPZ Challenge (NF54) administered by DVI to infect the participants. In the BSPZV2 trial conducted in Bagamoyo, Tanzania (#8 in **Table 2**), 6/6 (100%) participants receiving 3 doses of 9×10^5 PfSPZ of PfSPZ Vaccine (total dose 2.7×10^6 PfSPZ) at 0, 8 and 16 weeks were protected against homologous CHMI at 3-10 weeks after last dose of vaccine, whereas all 6 controls became infected [15]. Interestingly, as might be predicted from the results in malaria-naïve participants, a 50% higher dose of vaccine administered on the same schedule protected only 33% of participants – thus, beyond a certain

threshold, higher doses did not appear to be better, duplicating the results seen in malaria-naïve individuals. Then, in the Mali 2 trial (#9 in **Table 2**), 30/30 (100%) participants receiving 3 doses of 1.8×10^6 PfSPZ of PfSPZ Vaccine (total dose 5.4×10^6 PfSPZ) at 0, 8 and 16 weeks were likewise protected against homologous CHMI at 3 weeks after the last dose of vaccine, whereas 8/15 normal saline controls became infected (Sissoko and Healy, unpublished). In another key finding, 4/4 participants protected against a first CHMI conducted at 3 weeks after immunization in the BSPZV1 trial in Tanzania (#5 in **Table 2**), were again protected when undergoing repeat CHMI at 24 weeks [32]. The durability of protection shown in these malaria-exposed adults mirrored that seen in the VRC314 trial of malaria-naïve participants discussed above [10]: once protected, vaccines appear to maintain protection for at least 6-18 months in both malaria-naïve and malaria-exposed populations. Collectively, these results prove that in adult Africans, in whom PfSPZ Vaccine is less immunogenic for antibodies compared to malaria-naïve individuals, the vaccine can still be protective at the 100% level.

C. Clearance is required prior to immunization in order to reduce the immunosuppressive effect of latent parasitemia: Sanaria and collaborators have measured VE against naturally transmitted Pf malaria infection in 6 field trials of PfSPZ Vaccine (#4, 9, 10, 12, 16 and 19 in **Table 2**) and 2 field trials of PfSPZ-CVac in Africa (#7 and 10 in **Table 5** in the next section). In the five trials where parasitemia was cleared prior to the first vaccine dose, including four trials of PfSPZ Vaccine (MLSPZV1, MLSPZV2, BFSPZV1, MLSPZV4) and one trial of PfSPZ-CVac (MLSPZCV3), there was statistically significant VE during the 6 months after the last dose of vaccine. In the three trials in which clearance prior to the first vaccine dose was not done, including two trials of PfSPZ Vaccine (MLSPZV3 and KSPZV1) and one trial of PfSPZ-CVac (MLSPCV1), there was no statistically significant VE during the 6 months after the last dose of vaccine. We believe that this is due to the immunosuppressive effects of asexual blood stage parasites on the immune response to pre-erythrocytic stage parasites [33-35]. The most striking comparison is between MLSPZV3 and MLSPZV4 – both trials assessing the identical regimen in Malian adults in two consecutive rainy seasons:

Table 3. VE against Pf infection in MLSPZV3 and MLSPZV4

	PfSPZ Vaccine dose	Regimen	Study population	Time-to-event VE (1 – odds ratio)		Proportional VE (1 – risk ratio)	
MLSPZV3	9.0×10^5 PfSPZ	Days 1, 8, 29 (no clearance before V1)	Men and women	14%	P = 0.521	8.3%	P = 0.732
MLSPZV4	9.0×10^5 PfSPZ	Days 1, 8, 29 (clearance before V1)	Women	41%	P = 0.005	23%	P < 0.001

In the first trial, MLSPZV3, conducted without pre-vaccination clearance, there was no significant protection, whereas when the identical regimen was administered after parasite clearance in MLSPZV4, there was highly significant protection (Diawara and Healy, unpublished).

When all eight trials are considered together, the difference in protection between trials with and without pre-treatment is statistically significant (Fisher's exact test $p=0.018$). For this reason, all future

field trials of PfSPZ vaccines, if taking place in endemic areas where study participants may be chronically infected with malaria, will include treatment with a curative antimalarial drug regimen prior to the first vaccination.

D. PfSPZ Vaccine is safe and well tolerated in African infants and children: Four studies have been conducted in African children and infants (#8, #12, #14 and #17 in **Table 2**). In Tanzania after an initial age de-escalation / dose-escalation pilot study for safety, a 9.0×10^5 PfSPZ dosage regimen (3 doses at 8-week intervals) was compared in adults, teenagers, children and infants (BSPZV2 trial). The vaccine was safe and well tolerated in all age groups. As had been found in prior studies of African adults, the antibody responses to PfCSP in the Tanzanian adults were ~10 times lower than the antibody responses to the same immunization regimen in U.S. adults. However, the antibody responses to PfCSP in the African infants were ~10 times higher than in the Tanzanian adults and comparable to the antibody responses in US adults [36]. A similar study in Equatorial Guinea (EGSPZV2) was then conducted and showed similar results (Jongo unpublished, [15]). A third pediatric trial was conducted in western Kenya (KSPZV1). After an initial age de-escalation / dose-escalation pilot study, a larger Phase 2 component was initiated in which more than 300 infants age 5-11 months were randomized in double-blind fashion to received 3 doses of normal saline placebo or 4.5×10^5 , 9.0×10^5 , or 1.8×10^6 PfSPZ of PfSPZ Vaccine, with administrations at 0, 8 and 16 weeks. In both the age de-escalation and efficacy phases of this study, PfSPZ Vaccine was safe and well tolerated [37, 38]. In addition, it was demonstrated that DVI of PfSPZ Vaccine in infants and young children was feasible [39]. However, VE was not statistically significant at the primary 6-month endpoint, although this may have been due to the lack of clearance prior to immunization as discussed above. There was significant protection in the high dose group at 3 months, however, against both malaria infection and clinical malaria, likely mediated by antibody responses [38]. The conclusion from this study was that the infant immune system was poorly adept at generating the cellular immune responses needed for durable protection (none could be detected), and that alternative regimens of PfSPZ Vaccine should be explored in order to induce a stronger cellular response in this age group. The final pediatric study, LaSPZV1, was conducted in 2- to 10-year-olds in Gabon. The vaccine appeared safe based on blinded data, with unblinded results pending (Agnandji, unpublished).

E. PfSPZ Vaccine is safe in HIV positive Tanzanian adults: In a small trial, PfSPZ Vaccine was assessed in HIV positive adults and shown to be safe. However, when 5 study participants underwent homologous CHMI, none were protected, whereas 80% (4/5) of non-HIV-infected adults were protected (Jongo, unpublished data).

F. The regimen of 9.0×10^5 PfSPZ on Days 1, 8 and 29 is the most protective among leading regimens: The EGSPZV3 trial (#18 in **Table 2**) was designed to identify the best regimen among leading regimens from prior trials. Homologous CHMI in naturally exposed African adults was used to measure VE. Of the four regimens studied, the condensed, 3-dose regimen of 9.0×10^5 PfSPZ on days 1, 8 and 29 appeared superior to the other regimens (Jongo submitted) and has been down-selected for further development.

5. Upcoming studies with PfSPZ Vaccine: The next trials of PfSPZ Vaccine are provided in **Table 4**. All are using the Days 1, 8 and 29 down-selected regimen from the EGSPZV3 trial:

Table 4. Upcoming trials of PfSPZ Vaccine

Location	Trial Identifier	Number of Vaccinees / Controls	Participant Age Range (years)	Pf Exposure	CHMI / Follow-up period	Estimated Start Date	Funding Source
USA	USSPZV6	42/12	18-50	7G8 CHMI	2, 6 and 10 weeks	October 2021	DoD
Europe	TRAVSPZV1	160/60	18-50	7G8, NF54, MAL31 CHMI	10 weeks	March 2022	German Government
Indonesia*	IDSPZV1	248/124	18-45	Field	9 months	March 2022	DoD
Mali	MLSPZV5	134/134	6-10	Field	6 and 18 months	March 2022	NIH
Equatorial Guinea	EGSPZV4	800/400	2-10	Field	6 and 15 months	August 2022	BIMEP

* this is the current trial

The first two trials, USSPZV6 and TRAVSPZV1 will be conducted in malaria-naïve populations in the USA and EU, respectively. VE will be assessed by heterologous CHMI. These trial designs have been accepted by the EMA as supportive of licensure. The last two trials, MLSPZV5 and EGSPZV4, will be conducted in malaria-exposed children in Mali and Equatorial Guinea, respectively. VE will be assessed by exposure to naturally transmitted Pf malaria and the primary outcome variable will be Pf clinical malaria, with Pf infection a secondary outcome variable. These trials are designed to support FDA licensure, but have not yet undergone FDA review. Among the five trials, only the Indonesia trial will (1) assess VE in malaria-naïve study participants in the field; (2) compare the VE of PfSPZ Vaccine and PfSPZ-CVac in the field, and (3) assess cross species protection (assess protection against *P. vivax* by a *P. falciparum* vaccine). It will be important at the current stage of development to assess which of these two vaccination approaches is the most potent for inducing durable, sterile protection in the field. The Indonesia trial is therefore critical to the development of both PfSPZ Vaccine and PfSPZ-CVac.

1.2.2.2 PfSPZ Challenge (PfSPZ-CVac)

PfSPZ-Challenge without co-administration of a partner drug: The initial use of PfSPZ Challenge was for CHMI, and the availability of this “challenge in a bottle” opened a pathway for the conduct of CHMI studies in Africa, where mosquito-bite CHMI had not previously been done due to the expense and the dangers of importing *Anopheles stephensi*, the South Asia malaria vector traditionally used for CHMI. CHMI by injection of PfSPZ Challenge was first done in the USA and Europe, and then transitioned to Africa, and has proven to be very safe and well tolerated. As of 02 November, 2021, 1226 research participants have received injections with PfSPZ Challenge outside the context of its use for PfSPZ-CVac, and there have been no severe or serious adverse events (SAEs) linked to its administration. The product was first administered successfully by the ID route in studies in the Netherlands [40], Tanzania [41], and the USA [42]. During the same period it was administered

successfully by the intramuscular (IM) route in studies in England [43], Kenya [44], and Spain [45]. However, in a situation analogous to PfSPZ Vaccine, IV or DVI routes proved to be the most efficient and consistent. The decision to use DVI for PfSPZ Challenge was based on a trial in Germany, followed by a trial in Spain, that showed 100% infection of controls with a dose of only 3.2×10^3 PfSPZ of PfSPZ Challenge [45, 46]. DVI of 3.2×10^3 PfSPZ is now the gold standard dose used for CHMI and has been 100% infective in malaria-naïve adults undergoing first CHMI. PfSPZ Challenge has now been used to conduct 16 separate CHMI's in six different trials to test PfSPZ Vaccine efficacy in three countries in Africa, and additional 38 separate CHMIs in five different trials to assess innate and naturally acquired immunity in three different countries in Africa.

PfSPZ Challenge with co-administration of a partner drug (PfSPZ-CVac): As with the initial studies of immunizing with irradiated sporozoites, immunizing with non-attenuated, fully infectious sporozoites under cover of an antimalarial drug was first investigated using mosquito-bite administration. This process, called at the time “chemoprophylaxis with sporozoites” (CPS), or “infection-treatment vaccination” was first done in a seminal study conducted in malaria-naïve adults in the Netherlands. Roestenberg et al. reported 100% protection against homologous CHMI performed 10 weeks after the last of three “doses” of ~15 infectious mosquito bites administered to 10 healthy participants receiving CQ chemoprophylaxis as the partner drug [47]. When protected participants underwent a second CHMI 28 months after immunization, 4/6 (67%) were still sterilely protected, and the other two showed delayed onset of parasitemia, indicating the durability of protection [48]. The development of PfSPZ Challenge has allowed Sanaria to duplicate these results using injectable PfSPZ as the immunogen. This is done by combining DVI of larger doses than used for CHMI (e.g., 5×10^4 PfSPZ) with oral administration of a partner drug to kill the parasites *in vivo*, an approach denoted PfSPZ-CVac (chemoprophylaxis vaccination).

There have been 7 clinical trials of PfSPZ-CVac completed in malaria naïve individuals and 3 in malaria exposed individuals in Africa. As of 02 November, 2021, 395 participants in the USA, the Netherlands, Germany, Mali, and Equatorial Guinea have received 1178 doses of PfSPZ Challenge for PfSPZ-CVac immunization. These clinical trials are listed in **Table 5** below. Additional details are included in the PfSPZ-CVac Investigator's Brochure (IB).

Table 5. Chronological Listing of Trials of PfSPZ-CVac

Name	Site Institution	N	Route	Partner drug	# doses	Highest # PfSPZ	Interval between injections	Pf exposure	Best protection*
1. TIP-5 (NCT01728701) Sep 2012 (completed)	Nijmegen Radboud UMC	20	ID	CQ	3-4	7.5×10^4	4 weeks	CHMI (homol)	0% (0/20)
2. TUCHMI-002 (NCT02115516) Apr 2014 (completed)	Tübingen Inst for Trop Med	45	DVI	CQ or CQ + azithromycin**	3	5.12×10^4	4 weeks 2 weeks 5 days	CHMI (homol)	100% (9/9) 67% (6/9) 63% (5/8)
3. 15-I-0169 (NCT02511054) Nov 2015	Bethesda NIAID / LMIV	20	DVI	PYR + CQ or CQ	3	5.12×10^4	4 weeks	PYR: CHMI (homol) CQ:	PYR 22.2% (2/9) CQ

(completed)								CHMI (homol)	80% (4/5)
4. MALACHITE (NCT02858817) Nov 2016 (completed)	Tübingen Inst for Trop Med	21	DVI	atovaquone / proguanil	3	1.5x10 ⁵	4 weeks	CHMI (homol)	27% (3/11)
5. EGSPZV2 (NCT02859350) Nov 2016 (completed)	Equatorial Guinea Malaria Vaccine Initiative	20	DVI	CQ	3	1x10 ⁵	4 weeks	CHMI (homol)	55% (8/13)
6. DMID 11-0042 (NCT02773979) Jan 2017 (completed)	Seattle Group Health	21	DVI	CQ	3	1.024x10 ⁵	7 days 5 days	CHMI (homol)	0% (0/8) 75% (6/8)
7. MLSPZCV1 (NCT02996695) Apr 2017 (completed)	Mali Malaria Research & Training Center	62	DVI	CQ	3	2.048x10 ⁵	4 weeks	Field exposure	33.6%**
8. 17-I-0067 (NCT03083847) Jun 2017 (completed)	Bethesda NIAID / LMIV	51	DVI	PYR or CQ	3	2x10 ⁵	4 weeks	PYR: CHMI (homol) CHMI (heterol) CQ: CHMI (heterol)	PYR: 87.5% (7/8) 77.8% (7/9) CQ: 100% (6/6)
9. CVac-Tü3 2018-004523-36* May 2019 (completed)	Tübingen Inst for Trop Med	20	DVI	CQ	3	1.1x10 ⁵	1 st interval 5 days, 2 nd interval 23 days	CHMI (heterol)	77% (10/13)
10. MLSPZCV3 (NCT03952650) Jun 2019 (completed)	Mali Malaria Research & Training Center	260	DVI	PYR or CQ	3 plus boost at 1 year	4x10 ⁵	4 weeks	Field exposure	PYR: Yr 1: 39%** Yr 2: 50%** CQ: Not tested

CQ = chloroquine; PYR = pyrimethamine; * EudraCT number; ** 1 minus the hazard ratio

The major findings are described here:

1. First clinical trial of PfSPZ-CVac: The PfSPZ-CVac approach was first assessed via the ID route in a Phase 1 trial in the Netherlands (study #1 in **Table 5**) [40]. Immunizations were well tolerated in all participants, but there was no protection against CHMI. The experience with PfSPZ Challenge thus paralleled that with PfSPZ Vaccine: in order to be efficacious, injection needed to be administered via the IV or DVI route.

2. 100% protection was achieved after DVI administration: A second trial of the PfSPZ-CVac with CQ as the partner drug was performed in Germany, called TüCHMI-002, and in this case the PfSPZ were administered by DVI (study #2 in **Table 5**). Immunizations were well tolerated in all participants and neither unexpected nor severe adverse reactions were observed during the two weeks after injection, other than a single participant who was non-compliant with study procedures (see Risks below). Part A of the trial followed a dose escalation design, with three doses of PfSPZ administered at 4 week intervals to reproduce the approach of Roestenberg et al [47]. The high dose group received doses of 5.12x10⁴ PfSPZ of PfSPZ Challenge, and demonstrated 100% VE (9/9 vaccines protected) after homologous CHMI performed 10 weeks after the last immunization [13]. The same dosage regimen, 5.12x10⁴ PfSPZ every 4 weeks, was later assessed by a team from the Laboratory of Malaria

Immunology and Vaccinology (LMIV), NIAID, NIH (study #3 in **Table 5**), and 4/5 (80%) of participants were protected, demonstrating that these results could be replicated [14].

3. Protection was achieved using condensed regimens: In Part B of the TÜCHMI-002 trial, the span of the 5.12×10^4 PfSPZ regimen was truncated from 8 to 4 weeks by administering 3 doses every 2 weeks, and then further cut to 10 days by administering 3 doses every 5 days. VE against homologous CHMI at 10 weeks after the last dose in these two regimens was 67% and 63%, respectively [13]. It was encouraging that condensed regimens were protective, even if VE was lower than the 100% observed using 4-week intervals. It was proposed that higher doses of PfSPZ might compensate for the reduced efficacy in condensed regimens. Therefore, twice the dose of PfSPZ (1.024×10^5) was studied in a subsequent trial conducted in Seattle (Study #6 in **Table 5**), also administered as 3 immunizations with 5-day intervals, and protection indeed appeared to be higher, 75% (6/8), although it did not reach 100% [35]. Further progress in testing condensed regimens was made in the TÜCHMI-003 trial in Germany (Study #9 in **Table 5**), in which 1.1×10^5 PfSPZ were administered on days 1, 6 and 29. VE against heterologous CHMI conducted 12 weeks after immunization was 77% (10/13 participants protected) [49]. Of note, rather than providing a loading dose of CQ two days before immunization and then weekly maintenance doses thereafter, only three CQ administrations were done, a loading dose on the day of each immunization, prior to the injection of PfSPZ Challenge. As there were no CQ failures, this suggests that weekly maintenance CQ is not needed. The simplified TÜCHMI-003 regimen required only three clinic visits, compared to the 13 visits required for the traditional week 1, 5 and 9 regimen with weekly CQ administration. However, because efficacy appears diminished, the Indonesia trial will follow the traditional 8-week regimen.

4. Parasitemia was less well tolerated and VE dropped to zero when 7 day intervals were assessed: The Seattle trial studied 7-day intervals as well as 5-day intervals, reasoning that it would be convenient for vaccinees to come to the clinic on the same day each week, rather than every fifth day. 5.12×10^4 PfSPZ were administered as in the Germany and LMIV trials. Unexpectedly, there was no protection (0/8) [35]; in addition, there was a statistically significant increase in the severity of adverse events during the brief period of parasitemia that occurred 7, 8 and 9 days after injection of PfSPZ Challenge, with several participants experiencing grade 3 adverse events, including fever to 39.0°C and grade 3 fatigue, malaise, headache, chills, myalgia or arthralgia. One participant was discontinued from ongoing immunizations as a result. This finding was attributed to the fact that with 7-day intervals, the injection of the second dose of PfSPZ was superimposed on the parasitemia induced by the first injection, likely affecting cytokine and other inflammatory responses. Although the mechanism is not clear, this apparently led to the unanticipated increase in the severity of signs and symptoms associated with the parasitemia, and also suppressed the protective immune response. It is remarkable that shortening this 7-day interval by two days (injection of PfSPZ Challenge every 5 rather than every 7 days) largely reversed these effects in two different studies using 5-day intervals, and there was significant protection against CHMI (63% in Germany, 75% in Seattle, using 5.12×10^4 and 1.024×10^5 PfSPZ, respectively), and parasitemia was better tolerated. The 5-day intervals avoid the superimposition of new PfSPZ on existing parasitemia, and this may be the reason for the improved tolerability and VE.

5. The signs and symptoms associated with parasitemia can be minimized by using pre-treatment with anti-inflammatory drugs: Three doses of a still higher dose (2×10^5 PfSPZ) of PfSPZ Challenge, also with CQ as the partner drug, was assessed by the LMIV team at NIAID, NIH, in the 17-

I-0067 trial (study #8 in **Table 5**). The participants were administered ibuprofen or naproxen presumptively starting the morning of the 7th day following the first PfSPZ Challenge administration and continuing every 6 to 8 (ibuprofen) or 12 (naproxen) hours for 24-36 hours to prevent clinical manifestations associated with parasitemia. Indeed, this approach completely prevented Grade 3 adverse events despite the higher dose of PfSPZ. In addition, due to the rapid development of immunity following the first dose, no anti-inflammatory drugs were administered after the second and third doses, and no Grade 3 adverse events were observed [14]. The use of anti-inflammatory pre-medication after the first dose (and thereafter when needed) is planned for the Indonesia trial.

6. Liver active partner drugs prevent parasitemia but reduce VE: PfSPZ Challenge has been administered with partner drugs other than CQ, including azithromycin (TÜCHMI-002 trial, Part B, study #2 in **Table 5**), atovaquone / proguanil (Malachite trial study #4 in **Table 5**), and pyrimethamine (the 15-I-0169 trial, and more recently, the 17-I-0067 trial, studies #3 and 8, respectively, in **Table 5**). These drugs / drug combinations were designed to kill the parasites in the liver before they reached the bloodstream, thereby avoiding parasitemia and the attendant signs, symptoms and risks. Although it reduced parasite burden, azithromycin was not effective in preventing blood stage infection and its use was stopped (Mordmüller, submitted). Atovaquone/proguanil successfully prevented blood stage infection, but even at a dose of 1.5×10^5 PfSPZ of PfSPZ Challenge administered 3 times at 4 week intervals, there was only modest protection (2/10) (Borrmann, unpublished). 2×10^5 PfSPZ of PfSPZ Challenge administered with pyrimethamine proved to be safe and well tolerated, also with no breakthrough Pf blood stage infections, but in the initial 15-I-0169 study, which used doses of 5.12×10^4 PfSPZ, only 22.2% (2/9) of research participants undergoing homologous CHMI were protected [14]. The follow-on 17-I-0067 study increased the dose 4-fold to 2×10^5 PfSPZ and protection improved to 87.5% (7/8) against homologous CHMI, and was 77.8% (7/9) against heterologous CHMI. In the same trial, PfSPZ-CVac (CQ) protected 6/6 (100%) research participants against heterologous CHMI conducted at 12 weeks after immunization, the best protection ever recorded for a malaria vaccine [14]. Until a sufficiently protective regimen using a liver active drug is identified, PfSPZ-CVac will continue development with CQ serving as the gold standard partner drug.

7. PfSPZ-CVac is very well tolerated in malaria-exposed Africans, causing few, if any, signs and symptoms associated with parasitemia. Three trials of PfSPZ-CVac (CQ) have been conducted in Africa. A regimen consisting of 3 doses of 1.0×10^5 PfSPZ every 4 weeks was tested in Equatorial Guinea in 20 young adults (study #5 in **Table 5**). Homologous CHMI was performed a median of 15 weeks after vaccination, and showed 55% protection (8/13 vaccinees vs. 1/7 controls negative by qPCR) [50]. A similar three-dose regimen, but with 2.048×10^5 PfSPZ per injection rather than 1.0×10^5 PfSPZ, was administered to 31 adult participants in a field study in Mali, with 31 control participants receiving normal saline placebo (study #7 in **Table 5**). As in Equatorial Guinea, the regimen was very well tolerated, with few if any adverse events attributed to PfSPZ Challenge administration (Thera and Laurens, unpublished). The participants were followed for 24 weeks during the rainy season and incident malaria infections were measured using active and passive case detection. VE was 33.6%, but did not reach statistical significance in this trial, where pre-vaccination clearance of parasitemia was not performed. A similar study was done in Mali two years later with pyrimethamine as the partner drug (study #10 in **Table 5**). In this study, the dose of PfSPZ was increased still further to 4.0×10^5 PfSPZ. In addition, parasitemia was cleared prior to the first immunization, which had not been done for the first field study. The vaccine was well tolerated and VE was 39% by time-to-event analysis (statistically significant), and

after receiving a boost a year later, protection persisted through a second transmission season (VE 50%) (Sagara and Cook, unpublished).

8. Upcoming studies with PfSPZ-CVac: The next trial of PfSPZ-CVac is the current trial in Indonesian soldiers (see **Table 4**), where PfSPZ-CVac will be compared head-to-head with PfSPZ Vaccine. PfSPZ Challenge will be administered as three doses of 2.0×10^5 PfSPZ, as in the successful 17-I-0067 trial at LMIV. This appears to be the most appropriate dose for malaria-naïve individuals, whereas 4.0×10^5 PfSPZ appears better for those with a history of Pf exposure.

1.2.3 The importance and rationale for the clinical trial

Based on these clinical data, optimized regimens have been selected for PfSPZ Vaccine and PfSPZ-CVac for use in the Indonesia trial. Three doses of PfSPZ will be administered for each vaccination approach as described here:

PfSPZ Vaccine: The regimen 1, 8 and 29 days was selected for PfSPZ Vaccine administration because it provided 79% VE against heterologous CHMI in the MAVACHE trial in Germany, which equalled the 78% VE it provided against homologous CHMI in the same trial (Mordmüller, submitted). A regimen equally effective against homologous and heterologous parasites is preferable for protection in the field, where all naturally transmitted parasites will be heterologous to the vaccine strain. In addition, the pre-deployment window for immunization is not sufficient to use the longer PfSPZ Vaccine regimens studied in the past, such as the 0, 8 and 16-week regimen used in multiple earlier trials (see **Table 2**). The dose of PfSPZ per injection for PfSPZ Vaccine will be 9×10^5 PfSPZ administered by DVI, the same dose found to be optimal in the MAVACHE trial in Germany (Mordmüller, submitted) and also the same dose providing 100% protection against homologous CHMI in the BSPZV2 trial in Tanzania [15]. When higher doses were used in Tanzania, protection decreased sharply to 33% [15], with a similar finding in Warfighter 2 trial [31].

PfSPZ-CVac (CQ): The regimen 0, 4 and 8 weeks was selected for PfSPZ Challenge administration because this regimen has been the gold standard, protecting 9/9 (100%) participants against homologous CHMI in the TüCHMI-002 trial, a result that was confirmed independently in the LMIV 15-I-0169 trial, where 4/5 (80%) participants were protected, and in the LMIV 17-I-0067 trial, where 6/6 (100%) of participants were protected. When shorter intervals were tried in the TüCHMI-002 and TüCHMI-003 trials in Germany and in the 11-0042 trial in Seattle, protection against homologous CHMI decreased to below 100% [13, 35, 49]. The dose selected for PfSPZ-CVac is 2.0×10^5 PfSPZ per dose. This is higher than the doses used in TüCHMI-002, TüCHMI-003 and Seattle trials (5.12×10^4 PfSPZ, 1.1×10^5 PfSPZ and 1.024×10^5 PfSPZ, respectively) but the same as the dose in the more recent 17-I-0067 trial conducted by LMIV at NIH, which obtained 100% (6/6) protection against heterologous CHMI performed 12 weeks after immunization [14]. The precise regimen from the LMIV trial, three doses of 2.0×10^5 PfSPZ administered by DVI on weeks 0, 4 and 8 will be used in the Indonesia trial.

Using these optimized regimens, PfSPZ Vaccine and PfSPZ-CVac will be compared against corresponding normal saline control groups, measuring safety, tolerability, immunogenicity and vaccine efficacy against naturally transmitted Pf in Papua. This will constitute the first assessment of these two vaccine products for the prevention of Pf malaria in the field in the same clinical trial and the first trial of

either vaccine in Asia. A placebo group injected with normal saline (NS) will be included as a comparator for each vaccine, using a randomized, double-blind design so that incidence of malaria and frequency of adverse events can be accurately measured. It is imperative to know the unfettered Pf and Pv malaria primary attack rate in Papua and the unfettered Pv relapse rate on re-deployment to the home base in order to estimate VE, so the placebo group must be included. There is no comparator vaccine administered by DVI that would allow blinding; in addition, normal saline serves as an inert comparator allowing sensitive detection of even minor adverse events caused by the vaccine after each administration. There will be ongoing malaria exposure during deployment, which will affect immune responses to malaria, and a placebo group will also help to differentiate the immunological effects of ongoing exposure from the those of immunization with the vaccines. To further isolate the specific effects of PfSPZ-CVac immunization, the placebo group for PfSPZ-CVac will receive CQ on the same schedule as the vaccine group. For the statistical measurement of VE, each vaccine group will be formally compared to its corresponding placebo group.

The measured VEs will inform whether either vaccine is protective enough to proceed to Phase 3 testing in Asia, or whether further optimization of dosing regimens may be needed. PfSPZ-CVac has a major advantage as compared to PfSPZ Vaccine, which is the ability to confer high-level VE against homologous CHMI at a dose of PfSPZ that is 20% the dose of PfSPZ required to achieve the same VE using PfSPZ Vaccine. This increased potency decreases the cost of goods and will be an important consideration when immunizing large numbers of people during mass vaccination programs in Indonesia or elsewhere. On the other hand, PfSPZ-CVac involves the administration of fully infectious PfSPZ, and if the partner drug (e.g. CQ) is not taken properly, the vaccine recipient could develop clinical malaria with potentially serious consequences. Gaining better understanding of VE, potency, immunogenicity, safety and tolerability are all important outcomes anticipated from the trial. If the trial is successful, it will help to propel one or both vaccines to Phase 3 testing and licensure, and the vaccines can then be used to promote public health in Indonesia through enhanced malaria control, with the possibility of using them for malaria elimination campaigns.

The trial will also assess whether PfSPZ Vaccine and PfSPZ-CVac, both comprised of Pf sporozoites, will provide cross-species protection against Pv. If they do, then these vaccines can be used to control and eliminated Pv as well as Pf. If cross-protection is marginal or absent, then Sanaria will know that a separate development effort will be needed for PvSPZ-based vaccines. The opportunity to assess cross-species protection is a unique strength of the trial design, and heightens the importance of the trial in Indonesia, where both Pf and Pv menace the population.

1.3 Risks/Benefit Assessment

1.3.1 Known potential risks

1.3.1.1 PfSPZ Vaccine

PfSPZ Vaccine has been exceptionally safe and well tolerated in malaria-naïve adults in the USA and Europe, and in malaria-exposed adults, children and infants in Africa. The near absence of reactogenicity may reflect selection pressures in nature shaping the surface coat of PfSPZ so that hepatocytes can be located and invaded without inducing a symptomatic inflammatory response. The pruritic papules induced by mosquito bites are the result of injected mosquito saliva, not sporozoites,

as has been demonstrated in trials of PfSPZ Vaccine and PfSPZ Challenge injected ID and SC. These routes of administration do not cause local inflammation at the injection site, including after repeated dosing. The manufacturing process at Sanaria removes nearly all mosquito components, contributing to the apparent absence of reactogenicity.

Even large doses of PfSPZ (the equivalent of perhaps thousands of infectious mosquito bites) have been remarkably well tolerated. Doses as high as 2.7×10^6 PfSPZ have been given by DVI and as high as 2.2×10^6 PfSPZ have been given IM without apparent side effects. In all trials so far, the majority of vaccine recipients reported no or mild systemic AEs, with only a few classified as moderate in severity. Fever has only rarely been reported as a solicited AE and the few reports to date were generally attributed to other causes. The first completely analyzed and published data set on safety and tolerability from a placebo-controlled trial came from the first trial in Mali (#4 in **Table 2**) [16] which compared 47 true-immunized to 46 placebo-immunized participants using a randomized, double-blind design and DVI as the route administration for 5 doses of PfSPZ Vaccine. There were no differences in AE profiles comparing vaccine and placebo groups. This included local and systemic solicited AEs, unsolicited AEs and laboratory abnormalities [16]. Twelve additional randomized, double-blind, placebo-controlled trials and two randomized, double-blind, placebo-controlled pilot studies in African adults, children and infants, have also found no differences in the rates of any AEs between vaccine recipients and controls after unblinding (main trials #5, 7, 8, 9, 10, 12, 13, 14, 15, 16, 18, and 19 and pilot studies for trials #10 and 12 in **Table 2**) [15, 17, 32, 36-38, 50, 51](Jongo, submitted; Sirima and Laurens, unpublished; Diawara and Healy, unpublished, Mordmüller, submitted), with the exception of one study (#10 in **Table 2**), where arthralgia (but no other adverse events) was more common in vaccine than in placebo recipients (Sirima and Laurens, unpublished). This study, however, administered a dose of PfSPZ Vaccine three times higher, 2.7×10^6 PfSPZ, than will be used in Indonesia. Although not statistically significant, there was a similar finding in the MAVACHE trial, where the same high dose of PfSPZ, 2.7×10^6 PfSPZ, was administered to malaria-naïve adults and several participants appeared to have typical vaccine reactions the evening after the second dose, which was administered by DVI 7 days after the first dose. For example, one individual experienced fever, chills, sweating, myalgia, fatigue and vomiting the evening after the second vaccination, which resolved the next day, although these signs and symptoms could also have resulted from an undiagnosed viral infection. Since the second dose of PfSPZ Vaccine is being given +7 days after the first in the Indonesia trial, this will be an area of interest during the analysis.

Among all these studies, most revealing are the findings from the KSPZV1 trial in infants in western Kenya, where there appeared to be no age effect or dose effect in the rates of adverse events, after administering doses as high as 1.8×10^6 PfSPZ to infants and young children, a dose that is twice as high as that planned for adults in Indonesia [37, 38]. There also did not appear to be an increase in adverse events with repeated dosing (i.e. the rate of adverse events after second or third doses compared to the rate after first dose). It is thus not clear that PfSPZ Vaccine has caused *any* adverse reactions at doses as high as 1.8×10^6 PfSPZ in any individuals, whatever their age or history of prior malaria exposure. In addition, there have been no allergic reactions clearly linked to the vaccine in any of the 20 different clinical trials of PfSPZ Vaccine (**Table 2**).

Against this background of favorable safety data from five double-blind, placebo-controlled trials, two serious adverse events (SAE's) have occurred that were assessed as possibly related to the administration of PfSPZ Vaccine. In each case the potential association was based on timing without a

clear pathophysiological link established. Both of these SAEs occurred in the EGSPZV2 trial in Equatorial Guinea (#14 in **Table 2**), and led to halting of the study while the SAEs were reviewed by the Data and Safety Monitoring Board (DSMB). Direct causality could not be established, and in each case the DSMB, all associated IRBs, the regulatory agency in Equatorial Guinea and the US FDA approved resumption of the trial.

In the first case, a 19-year old woman became pregnant at about the same time as she received a first dose of 2.7×10^6 PfSPZ of PfSPZ Vaccine. An ultrasound at 9 weeks gestational age (by last menstrual period) showed a 6 week sized embryo without cardiac activity. Because about a quarter to a third of pregnancies end in spontaneous abortion, it is unclear whether the administration of PfSPZ was related to the loss of this embryo. As was the case in the EGSPZV2 trial, avoidance of pregnancy remains an important inclusion criterion for trials of PfSPZ Vaccine, although Sanaria is developing a program for testing the vaccine in pregnant women. Since the trial in Indonesia will be enrolling only male soldiers, pregnancy will not be a concern.

In the second case, a 15-year old boy with no known history of seizures experienced a seizure $3\frac{1}{2}$ hours after receiving a third dose of 1.8×10^6 PfSPZ of PfSPZ Vaccine. A work-up showed that the boy had a normal head CT scan and a normal head MRI. However, an electroencephalogram (EEG) revealed abnormalities consistent with generalized epilepsy. Consulting neurologists posited that the general inflammatory response to the vaccine may have lowered the seizure threshold in this individual, whose EEG is consistent with a predisposition to seizures, and whose sister has epilepsy, although the precise mechanism that could explain the link is not clear. At this point, it is not possible to know whether the PfSPZ non-specifically affected the seizure threshold in this boy, or whether the timing of the seizure was coincidental. This is the only seizure potentially associated with PfSPZ Vaccine. The same dose of PfSPZ Vaccine has been administered by DVI to 150 other children ranging in age from 5 months to 17 years, including 92 infants, most of whom received three injections with this dose, without any identifiable adverse reactions. However, as a precaution, Sanaria recommends excluding any individual with a history of non-febrile seizures from participation in clinical trials of PfSPZ-based products, although with further clinical experience this restriction may be lifted. This exclusion applies to the trial in Indonesia.

Although the data indicate that PfSPZ Vaccine does not cause adverse reactions at the doses planned for the Indonesia study, nevertheless the consent form lists the typical reactions any vaccine might theoretically cause, including:

Local reactions: injection site pain, erythema, swelling, induration, pruritus, bleeding, and arm motion limitation.

Systemic reactions: fever, chills, malaise, fatigue, irritability, headache, dizziness, myalgia, nausea, vomiting, regional lymphadenopathy, difficulty sleeping, anxiety, confusion, cough, shortness of breath, palpitations, and allergic reactions such as urticaria, pruritus, edema, rash and anaphylaxis, the latter potentially resulting in death, and additionally unexpected adverse events that have not previously been reported.

1.3.1.2 PfSPZ-CVac (PfSPZ Challenge + chloroquine)

Risks associated with PfSPZ: Immunization with PfSPZ-CVac has the same benign safety profile during the initial six days following injection of PfSPZ Challenge as does PfSPZ Vaccine, locally at the site of injection, and systemically. The TÜCHMI-002 PfSPZ-CVac trial in Germany included blinded placebo controls, and there was no difference in adverse event profiles between vaccine and placebo recipients (#2 in **Table 5**) [16]. The same is true in the trials of PfSPZ-CVac in Mali (#7 and #10 in **Table 5**) and Equatorial Guinea (#5 in **Table 5**). In none of these studies was there any difference in the rates of adverse events in vaccinees and placebo recipients when compared over the first six days after immunization ([50]; Thera and Laurens, unpublished; Sagara and Cook, unpublished).

The adverse event profile of PfSPZ-CVac changes after six days, however, due to the exiting of the parasites out of the liver into the blood. As discussed previously, injection with PfSPZ Challenge induces transient parasitemia in recipients when the approach relies on a blood schizonticide such as CQ as the partner drug. This transient parasitemia occurs +6 to +10 days after injection, reaching maximum density on +7, +8 or +9 days, and then falls quickly, as the parasites are immediately exposed to therapeutic levels of CQ, and are rapidly killed. In the Tübingen TÜCHMI-002 trial (#2 in **Table 5**), the highest density recorded after administering 5.12×10^4 PfSPZ of PfSPZ Challenge by DVI was 36.4 parasites/ μ L. The geometric mean density in all 9 participants after the first dose of PfSPZ Challenge was 15.7 parasites/ μ L. When highly sensitive quantitative polymerase chain reaction (qPCR) was used for detection, parasite densities fell by day 10 to a very low (~ 0.01 parasite/ μ L) or undetectable level. Any residual low reading on qPCR appear to represent detection of nucleic acid from non-viable parasites, since recrudescence infections have not occurred in these individuals.

The highest parasite densities seen in the TÜCHMI-002 trial were high enough to cause symptoms in some study participants, since the symptom threshold is typically crossed at densities of 5-15 parasites/ μ L, although others may not experience symptoms until parasite densities exceed 50 parasites/ μ L. Altogether, 4/18 (22.2%) research participants immunized with doses of 1.28×10^4 or 5.12×10^4 PfSPZ in this trial experienced at least one grade 3 (severe) adverse event during the period of transient parasitemia; all of these severe malaria-related signs and symptoms resolved spontaneously [13].

Grade 3 adverse events during the period of transient parasitemia have since been reported in other PfSPZ-CVac (CQ) trials in malaria-naïve individuals. In the Seattle trial, for example, where condensed regimens were tested (#6 in **Table 5**), roughly 25% of research participants receiving PfSPZ-CVac (CQ) experienced Grade 3 adverse events and three were withdrawn from further vaccinations as a result [35]. As discussed earlier, the frequency of Grade 3 adverse events in this trial may have been in part related to the short intervals between doses (5 or 7 days), as side effects have appeared less severe when the gold standard regimen is used, with dosing every 4 weeks. With this longer regimen, grade 3 adverse events are generally not seen after the second and third doses, presumably because the research participants develop a degree of immunity after the first dose. This hypothesis is consistent with the findings from trials of PfSPZ-CVac (CQ) conducted in malaria-exposed individuals in Africa. Naturally acquired immunity appears to prevent any signs or symptoms of malaria on days +6 to +10 after PfSPZ-CVac (CQ), as shown by two different trials of PfSPZ-CVac (CQ) in malaria-exposed African adults ([50]; Thera and Laurens, unpublished).

Additional experience has been gained with malaria-related side effects of PfSPZ-CVac (CQ) in two studies performed by LMIV at the NIH Clinical Center (#3 and #8 in **Table 5**). The first trial used

doses of 5.12×10^4 PfSPZ, the same dose used in the Germany and Seattle trials, and the second approximately 4-fold higher doses of 2.0×10^5 PfSPZ. Because grade 3 adverse events were recorded in the first trial, the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen and naproxen were used in the second trial. Treatment was administered for two days after the first PfSPZ Challenge injection starting the morning of day +7. This completely prevented any Grade 2 or Grade 3 adverse events despite the 4-fold higher dose. Consistent with rapid onset of immunity after the first immunization, no participant needed further NSAID administration after the second and third doses [14].

Based on this experience, NSAIDs will be used in all research participants after the first immunization with PfSPZ-CVac or placebo in the Indonesia trial. Treatment will be given presumptively the morning of the seventh day (using DOT), and study participants will take doses later in the day and the following day on their own. The administration of NSAIDs, taken with the prolonged dosing regimen of PfSPZ-CVac in this trial, leads us to believe that few, if any, participants will experience a grade 3 adverse event.

Given the fact that parasitemia occurs during PfSPZ-CVac immunization, administering the partner drug is critical for maintaining the safety of the study participants. CQ has been selected due to the high efficacy of PfSPZ-CVac when this partner drug is used (see the description of VE earlier in the protocol), and because the NF54 parasite strain is highly sensitive to this drug. The 50% inhibitory concentrations (IC50s) for the PfSPZ Challenge Master Cell Bank (MCB) and Working Cell Bank (WCB) are 8.6-8.7 ng/mL, and this cannot change (i.e., resistance cannot evolve) because each lot of PfSPZ Challenge manufactured by Sanaria is derived from the same WCB. When these IC50s are compared to the lowest CQ level ever measured to date in a trial of PfSPZ-CVac, 32 ng/mL, it can be seen that there is a large safety margin. CQ levels are generally much higher than this, ranging upward to 110 ng/mL. Safety is increased by the fact that CQ is metabolized to desethyl-CQ, which also has parasitocidal activity against asexual blood stages. It is thus not surprising that, with one exception (see below), no recrudescence infections have occurred in any research participant during PfSPZ-CVac immunization.

To assure the proper administration of CQ to all participants, directly observed treatment (DOT) will be done in the Indonesia trial. Equally important to assure safety is to monitor for signs and symptoms of parasitemia after immunization, especially days 7-10 after each injection of PfSPZ Challenge. Daily clinical assessment during this period will be done in the Indonesia trial. If signs or symptoms likely caused by parasitemia occur, they will be followed closely. Thick blood smears will be checked as indicated (minimally on the seventh, eighth and ninth days after PfSPZ Challenge injection), and daily follow-up will continue until resolution, with re-treatment instituted with another antimalarial drug or drug combination only if clinically indicated.

No SAEs deemed related to PfSPZ Challenge have occurred in any trials of PfSPZ-CVac. However, two noteworthy AEs have occurred, and merit description. The first occurred in the TIP5 trial (#1 in **Table 3**) where PfSPZ Challenge was administered by ID injection. Several hours after the fourth CQ dose, one participant experienced transitory urticaria at multiple sites of the body lasting for 3 days (corresponding to +5 to +8 days after PfSPZ Challenge injection). The participant did not receive any treatment for the urticaria, continued in the study, received two more injections with PfSPZ Challenge and underwent CHMI, but did not develop recurrent urticaria or any other indication of an allergic reaction. The etiology of the urticaria was unclear. The second noteworthy adverse event was an SAE that occurred +3 days after immunization #3 in the 15-I-0169 trial (# 3 in **Table 3**). A 23-year-old

participant in a group receiving the antimalarial drugs pyrimethamine and CQ presented with the acute onset of nausea, vomiting, headache, tinnitus, mental “fogginess” and confusion worsening over the course of a week. The participant was hospitalized for 4 days to facilitate evaluation and management, and treated with IV acyclovir for presumed herpes simplex virus encephalitis. Cerebrospinal fluid was not obtained, because the participant had a Chiari malformation noted on brain MRI scan. Symptoms improved over time with the main symptom of “fogginess” fully resolving in 11 days. Convalescent viral titers were unrevealing. Final diagnosis was encephalopathy of unknown etiology deemed possibly related to CQ, which has been reported to cause a similar clinical picture, and unrelated to PfSPZ Challenge.

In addition to these two adverse events, there was an unanticipated event that placed a study participant at risk and was reported to the Safety Monitoring Committee (SMC), the IRBs and the US and German regulatory agencies. This occurred during the second part of the TÜCHMI-002 trial (#2 in **Table 3**), during follow-up after the first immunization. The research participant experienced parasitemia documented by PCR on +7 days after PfSPZ Challenge injection, with the parasite burden peaking on +8 days at a density by qPCR (15.7 parasites/uL) that was typical for the study. Parasitemia then fell progressively +9 and +10 days after PfSPZ Challenge, consistent with natural cycling of blood stage parasite growth and/or partial killing by CQ. However, there was increasing parasitemia on +11 days post PfSPZ Challenge by qPCR, and the participant was found to be thick blood smear positive on +13 days, and experienced mild symptoms of malaria (headache, sweating), suggesting that sufficient CQ was either not ingested or not absorbed. Parasitemia peaked on +13 days post PfSPZ Challenge (1271.2 parasites/uL). The participant was treated with atovaquone / proguanil on +13 to +15 days post PfSPZ Challenge and parasitemia cleared promptly.

CQ was detectable in this participant’s plasma on +13 days post PfSPZ Challenge, but was less than 2 ng/mL (limit of assay quantification 5 ng/mL). On questioning, the participant stated that he did swallow the CQ tablets. Notably, oral inspections post CQ ingestion were not performed in this trial. The participant initially agreed but later refused to undergo a pharmacokinetic study of CQ absorption and metabolism to rule out poor bioavailability as the cause of the very low CQ levels. However, since bioavailability of CQ is generally excellent (75-100%), poor bioavailability in this healthy participant seemed unlikely as the cause. The most likely explanation is surreptitious avoidance of CQ ingestion – hiding the tablets in the cheek and spitting them out later, or self-induced emesis after swallowing. Brief exposure of the oral or gastric mucosa to the drug in this fashion and subsequent absorption of small amounts would explain the trace levels measured in the blood and the initial drop in parasitemia on +9 and +10 days post PfSPZ Challenge. The parasite from this participant was isolated on +13 days post PfSPZ Challenge and tested *in vitro* for CQ susceptibility, and was found to be highly sensitive, consistent with the testing that has been performed on Sanaria’s MCB and WCB for PfSPZ Challenge. Since this event, Sanaria has recommended procedures to make sure the tablets have been swallowed.

Risks associated with the partner drug: Research participants receiving PfSPZ-CVac may experience the side effects of the partner drug. For example, CQ is well known to cause headaches, malaise, dizziness, nausea, vomiting, abdominal pain, tinnitus, hearing loss, blurred vision, photosensitivity, muscle weakness, insomnia, pruritus, anxiety, and confusion. When these side effects have occurred, they have generally disappeared even with ongoing use of the drug, and have always reversed after administration was stopped. A serious adverse reaction to CQ, has been seen once (a serious adverse event or SAE deemed “possibly related”) – the case of transient

encephalopathy described above, seen in a trial conducted at the NIH (#3 in **Table 5**) [14]. Other known serious side effects of CQ, such as seizures or life-threatening allergic reactions, have not occurred, although they remain theoretically possible. Because prophylactic doses rather than treatment doses of CQ are administered, CQ side effects during immunization with PfSPZ-CVac are generally minimal.

Summary: In summary, although the data indicate that PfSPZ Challenge does not cause adverse reactions at the doses planned for this study during the first 6 days after injection, nevertheless the consent form lists the typical reactions any vaccine might theoretically cause, including:

Local reactions: injection site pain, erythema, swelling, induration, pruritus, bleeding, and arm motion limitation, bruising / extravasated blood and pruritus.

Systemic reactions: fever, chills, malaise, fatigue, irritability, headache, dizziness, myalgia, arthralgia, nausea, vomiting, diarrhea, regional lymphadenopathy, difficulty sleeping, anxiety, confusion, cough, chest pain, shortness of breath, palpitations, and allergic reactions such as urticaria, pruritus, edema, rash and anaphylaxis, the latter potentially resulting in death, and additionally unexpected adverse events that have not previously been reported.

Additionally, the consent form lists the signs and symptoms associated with malaria parasitemia that may be experienced +7 to +10 days after each injection of PfSPZ Challenge, including fever, chills, sweats, rigors, malaise, fatigue, headache, myalgias, arthralgias, nausea and vomiting. These signs and symptoms could reach grade 3 in severity, but are always rapidly resolving. Grade 3 signs and symptoms of malaria associated with transient parasitemia will not lead to pausing of the study unless reaching a predetermined frequency (see pausing rules).

Finally, the consent form lists the additional side effects that can be caused by CQ, including such as headache, malaise, dizziness, nausea, vomiting, abdominal pain, tinnitus, hearing loss, blurred vision, photosensitivity, muscle weakness, insomnia, pruritus, anxiety, and confusion. In addition, CQ may cause seizures or life-threatening allergic reactions. If these or other adverse reactions known to be associated with CQ occur, even if they are serious (leading to hospitalization), this will not mandate pausing the study. Rather, CQ will be immediately discontinued in the affected study participant, any further treatment needed will be given, and no further PfSPZ Challenge injections will be administered to this individual.

1.3.1.3 Dihydroartemisinin/Piperaquine (DHA-PP)

DHA-PP is the primary antimalarial drug combination recommended by the Indonesian Ministry of Health and will be the first line antimalarial used for treatment of Pf and Pv (or any other species) malaria. In Papua, Indonesia, where chloroquine-resistance is common in both Pf and Pv, DHA-PP is effective against both Pf and Pv [52]. Treatment courses with DHA-PP are generally very well tolerated. The adverse effects reported included nausea, diarrhea and vomiting, as well as anorexia, anaemia, dizziness, headache, sleep disturbance and cough [53]. Although there has been no evidence of cardiotoxicity in large randomized trials and with extensive use of DHA-PP, PP does prolong the QT interval on electrocardiography by approximately the same amount as chloroquine. Significant prolongation of the QTc interval may cause potentially life-threatening ventricular tachyarrhythmia, but

there is no evidence that this has occurred with piperazine, despite its extensive use [53]. Nevertheless, DHA-PP should not be used in patients with congenital QTc prolongation or who have a clinical condition or are on medication that results in QTc interval prolongation. Potential participants who have prolonged QTc intervals will be screened out from participation in this clinical trial (see Exclusion Criteria).

Neither DHA nor PP have any demonstrable effect on sporozoites or liver stage parasites, and thus should not affect the induction of immunity by the PfSPZ in PfSPZ Vaccine or PfSPZ Challenge. PP is a 4-aminoquinoline, like chloroquine, and showed an equivalent lack of pharmaceutical effect on HepG2 (liver) cell invasion by freshly dissected *Plasmodium yoelii* sporozoites [54].

The elimination half-life of DHA is about 1 hour and of piperazine 2 to 4 weeks.

1.3.1.4 Venipuncture for direct venous inoculation and blood tests

DVI: DVI is the preferred route for administering Sanaria's PfSPZ products. The risks of DVI are the same as for any other venipuncture of a superficial arm vein: discomfort, bleeding, hematoma, infection, and vasovagal response. In the DVI procedure, a small-bore (25 gauge) needle is inserted into the vein, the syringe plunger is withdrawn slightly to demonstrate blood flashback, and the vaccine injected directly, a procedure lasting <10 seconds once the skin has been prepped with alcohol. As of 02 November 2021, 2771 adults, children and infants have received 7788 DVI injections of PfSPZ Vaccine, PfSPZ Challenge alone or PfSPZ Challenge as part of PfSPZ-CVac, or PfSPZ-GA1. The procedure has been uniformly well tolerated. In Tanzania and Equatorial Guinea (#5 and #7 in **Table 2**), recipients were asked immediately after the procedure to subjectively evaluate the level of pain as no pain, mild pain, moderate pain or severe pain, and more than 95% selected no pain (**Table 6**). This is likely due to the small needle (the trauma of penetration is minimized) and the fact that with intravascular injection there is no depot of fluid forced into a tissue space as with other routes of injection. Clinical investigators have remarked on the atraumatic nature of DVI injections compared with other parenteral routes of vaccine administration.

Table 6.
Subjective
rating of the
pain of
injection by
DVI in two
African Trials.

Number of Injections	Equatorial Guinea		Tanzania				Combined
	Vaccine		CHMI				
	48		277		52		
Pain	Number	Percent	Number	Percent	Number	Percent	Percent
None	46	95.8	269	97.1	52	100.0	97.3
Mild	2	4.2	7	2.5	0	0.0	2.4
Moderate	0	0.0	1	0.4	0	0.0	0.3
Severe	0	0.0	0	0.0	0	0.0	0.0

Blood Tests: The risks of blood drawing for obtaining blood samples for blood tests are the same as for DVI – discomfort, bleeding, hematoma, infection, and vasovagal response.

The risks of DVI and blood tests will be minimized by using trained personnel for the procedure, aseptic technique, single use needles and syringes, application of pressure on the puncture site after withdrawing the needle, and a low threshold for placing study participants in a supine position if they

feel light-headed. The total amount of blood withdrawn in any 8-week period during this trial will be well below the maximum of 525 mL allowed by blood banks.

To mitigate the risks of allergic reaction, including those that may be serious or life threatening, a study doctor will be present to monitor the vaccination process and the participants for at least 30 minutes after vaccination. Emergency equipment and supplies, including epinephrine, diphenhydramine, and prednisone, will be available to treat acute allergic symptoms as per standard medical procedures.

1.3.1.5 Malaria infection in the field

The risk of acquiring acute malaria at the field study site in eastern Indonesia is extremely high. Indeed, that risk makes the Indonesia trial feasible. Study participants will not be offered chemoprophylaxis against malaria, because this is the standard of care of the Indonesian Army. Military medical doctrine is to practice prompt diagnosis and treatment as their strategy to mitigate the malaria hazard. The research team will use the same approach, and is well trained through years of experience to manage clinical malaria in a manner that assures prompt diagnosis and effective treatment each and every time that it occurs.

If not diagnosed and treated, malaria in non-immune people like the Indonesian trial study participants may rapidly progress to severe and life-threatening syndromes like seizures, coma, respiratory distress, hepatic or renal dysfunction, and shock. The research team is acutely aware of this hazard and commits to the necessity of close and constant monitoring for the onset of malaria at its earliest stages and immediate chemotherapy with DHA-PP which is approved and recommended for use in Indonesia.

1.3.1.6 Loss of confidentiality

There is a potential risk of loss of confidentiality to study participants. Private health information recorded on participant case report forms could theoretically become available to non-study personnel. To protect against this potential risk, the principal investigator will carefully monitor study procedures to protect the confidentiality of participants and the quality of the data. Biological samples will be labeled with participant ID number rather than personal identifiers like name or date of birth. The key to this number (linking it to a named person), and all private health information will be kept in a locked cabinet in a locked room that is accessible only to authorized study personnel.

1.3.1.7 Study personnel

The main risks to study personnel at the Jakarta and home base study sites are from accidental exposure to blood and body fluid-borne infections. SOPs for staff safety will be used in clinical and laboratory areas, including sharps management, hazardous waste management, etc. Universal precautions will be used for handling all body fluids.

The study personnel posted with the battalion in malarious eastern Indonesia not only face these hazards, but face as well the very significant risk of acquiring malaria. All team members will be informed of this risk and offered the option of clinically prescribed chemoprophylaxis against malaria. Alternatively, team members may consume presumptive DHA-PP therapy (full treatment dose) each month.

Regardless of chemoprophylaxis choices, all team members will be under close monitoring for signs and symptoms of malaria. Team members are already acquainted with the symptoms of early malaria. Any illness occurring (to include that considered atypical for malaria) will prompt an expert microscopic examination for evidence of infection by the parasites causing malaria. In the event of confirmed acute malaria, team members will be provided with effective chemotherapy and clinical monitoring until recovery. Team members will also be provided with high quality insecticide-treated bed nets at their living quarters, in addition to being instructed on personal protection measures against biting mosquitoes (repellents, long-sleeved shirts and long pants, covered shoes, etc.).

1.3.1.8 Known potential benefits

The problem of malaria in Indonesian soldiers in the conduct of their duties is a serious threat and access to an effective vaccine would be a very substantial benefit. However, it is not yet known if either PfSPZ Vaccine or PfSPZ-CVac will be effective in the Indonesian soldier population, particularly as the strains of Pf malaria in eastern Indonesia are antigenically different than those in Africa, and neither product has yet been tested in Asia. Placebo recipients will not receive any benefits from normal saline injections.

Participation in the trial, however, brings a direct, tangible, and important benefit to every member of the battalion whether enrolled in the study or not: relatively intensive monitoring for signs of malaria and immediate diagnosis and treatment in an area where infection is highly likely and quite dangerous if not promptly diagnosed and effectively treated. The research team provides this intensive monitoring and prompt treatment services 24/7 to all members of the battalion while they are in eastern Indonesia. The benefit provided by the presence of the research team is greatly diminished risk of a poor health outcome due to malaria or other infections while in a high-risk environment.

These medical benefits extend to all soldiers in the battalion. All will receive outpatient follow-up medical care at the study sites at the home base and in eastern Indonesia without prejudice regarding enrollment status. Medical care for ailments not related to vaccination will not extend beyond the study follow-up period. Medical care for ailments related to vaccination will extend, at minimum, until the condition has resolved or stabilized.

2 STUDY OBJECTIVES

2.1 Primary Objectives

- 1) To assess the safety and tolerability of PfSPZ Vaccine and PfSPZ-CVac compared to placebo in Indonesian soldiers.
- 2) To assess the protective efficacy (vaccine efficacy = VE) of PfSPZ Vaccine and PfSPZ-CVac against **first clinical malaria cases** caused by *P. falciparum* (Pf) identified by thick blood smear (TBS) microscopy or rapid diagnostic testing (RDT) in naturally exposed Indonesian soldiers.

2.2 Secondary Objectives

- 1) To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against **first infections** caused by Pf identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers.
- 2) To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against **first clinical malaria cases** caused by *P. vivax* (Pv) identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers.
- 3) To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against **first infections** caused by Pv identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers.
- 4) To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against **relapsing infection** from latent liver stages of Pv identified post-exposure in a malaria-free area.
- 5) To identify humoral immune responses that predict VE of PfSPZ Vaccine and/or PfSPZ-CVac.

Exploratory:

- 1) To assess the VE of PfSPZ Vaccine and PfSPZ-CVac against **all cases of clinical malaria** caused by Pf and Pv identified by TBS microscopy or RDT in naturally exposed Indonesian soldiers.
- 2) To assess the VE of PfSPZ Vaccine and PfSPZ-CVac for reducing the number of asymptomatic Pf and Pv malaria infections.
- 3) To identify cellular immune responses that predict VE of PfSPZ Vaccine and PfSPZ-CVac.
- 4) To identify transcriptome or biomarker (protein) signatures that predict VE.
- 5) To identify markers of latent infection with Pv.

2.3 Study Outcome Measures

2.3.1 Case Definitions

Clinical malaria (primary endpoint if caused by Pf, secondary if caused by Pv) is defined as:

A positive thick blood smear at any density *plus*

- measured axillary temperature ≥ 37.5 degrees Celsius or history of fever in the last 24 hours; or
- at least two of the following symptoms/symptom groups: headache; chills and/or rigors; malaise and/or fatigue; dizziness and/or light-headedness; myalgias and/or arthralgias;
- or
- meeting criteria for severe malaria.

Malaria infection (secondary endpoint if caused by Pf or Pv) is defined as:

A positive thick blood smear at any density.

2.3.2 Primary outcome measures

- 1) The number of adverse events occurring after investigational product (IP) administration (the rates in vaccinees will be compared to the rates in placebo recipients):
 - a) The proportion of participants experiencing of solicited AEs occurring within 7 days (PfSPZ Vaccine) or 14 days (PfSPZ-CVac) of each administration of investigational product (IP)
 - i) AEs after each dose will be tallied separately, but a given AE will only be counted once after a given dose if it occurs repeatedly after that dose.
 - ii) For PfSPZ-CVac, systemic AEs will be tallied separately for days 1-6 after vaccination (AEs caused by sporozoites/liver stages), days 7-10 after each vaccination (AEs caused by blood stages), and for days -1 and -2 in combination with the period from day 11 after each vaccination to the next vaccination or, in the case of the third vaccination, from day 11 until the last AE collection after the last chloroquine administration (AE's caused by chloroquine in the absence of sporozoite, liver or blood stages).
 - b) The proportion of participants experiencing serious adverse events (SAEs) deemed related to IP during active participation in the trial.
 - c) The proportion of participants experiencing unsolicited AEs occurring within 14 days of each administration of IP deemed related to vaccination or placebo administration.
- 2) The number of **first clinical malaria cases** caused by **Pf*** among participants receiving vaccine vs. placebo during the period from 10 days after arriving in eastern Indonesia through the last day before leaving eastern Indonesia that the participant is accessible to the clinical team (diagnoses beyond that point cannot be made by microscopy).

* as an exploratory objective, RDT results will be retrospectively confirmed by microscopy or qPCR and microscopy results will be retrospectively confirmed by qPCR, by using, if possible, the FDA-approved biomarker assay developed by the laboratory of Dr. Sean Murphy, University of Washington Medical Center (the algorithm for confirmation will be specified in Statistical Analysis Plan).

2.3.3 Secondary outcome measures

- 1) The number of **first infections*** caused by **Pf**** among participants receiving vaccine vs. placebo during the period from 10 days after arriving in eastern Indonesia through 10 days after leaving eastern Indonesia.

* thick blood smears from asymptomatic individuals will be read retrospectively, so there is no interference in ascertaining the number of clinical cases, which is the primary VE endpoint. ** Same exploratory objective involving confirmed cases or infections as above.
- 2) The number of confirmed **first clinical malaria cases** caused by **Pv*** among participants receiving vaccine vs. placebo during the period from 10 days after arriving in eastern Indonesia through 10 days after leaving eastern Indonesia. * Same exploratory objective as above.

- 3) The number of confirmed **first infections*** caused by **Pv**** among participants receiving vaccine vs. placebo during the period from 10 days after arriving in eastern Indonesia through 10 days after leaving eastern Indonesia.
 * thick blood smears from asymptomatic individuals will be read retrospectively, so there is no interference in ascertaining the number of clinical cases, which is the primary VE endpoint. ** Same exploratory objective as above.
- 4) The number of confirmed **relapsing clinical infections** from latent liver stages of **Pv** identified post-exposure in a malaria-free area.
- 5) (a) The humoral immune responses* induced by vaccination compared to those induced by placebo administration comparing vaccinees to controls; (b) the association between the immune responses and protection (no parasitemia or clinical malaria occurring during surveillance).
 * (i) Levels of antibodies against Pf circumsporozoite protein (CSP) by ELISA 2 weeks after the third dose of vaccine;
 (ii) Optional: Levels of antibodies against PvCSP by ELISA 2 weeks after the third dose of vaccine;
 (iii) Optional: Levels of antibodies against other Pf and Pv proteins, PfSPZ, PvSPZ and Pf and Pv asexual erythrocytic stages (AES) 2 weeks after the third dose;
 (iv) Optional: inhibition of sporozoite invasion assay.

Exploratory (optional):

- 1) The total number of confirmed **clinical malaria cases** caused by **Pf** and **Pv** among participants receiving vaccine vs. placebo during the period from 10 days after arriving in eastern Indonesia through 10 days after leaving eastern Indonesia.
- 2) The total number of asymptomatic **Pf** and **Pv** malaria infections as measured by the number of four week intervals during deployment where TBS was positive and the participant remained asymptomatic for at least 14 days (positive TBS are discounted if the participant developed a symptomatic infection with the same species within two weeks) (four week intervals are counted from the time of each automatic TBS obtained in an asymptomatic participant).
- 3) (a) Cellular immune responses* against PfSPZ, PvSPZ and/or Pf AES prior to vaccination and 2 weeks after the third dose of vaccine comparing vaccinees to controls; (b) the association between the immune responses and protection (no parasitemia or clinical malaria occurring during surveillance).
 * Cellular immune responses to whole PfSPZ or PvSPZ and synthetic peptides from selected Pf and Pv antigens by intracellular cytokine staining (ICS)/ flow cytometry and/or other assays to be defined.
- 4) (a) RNA transcriptome quantification as detected by RNA-seq and protein levels in serum, and serum analyte analysis; (b) the association between these results and protection (no parasitemia or clinical malaria occurring during surveillance).
- 5) Serum samples will be used to probe protein microarrays developed by the Walter and Eliza Hall Institute, Melbourne; these arrays include a large number of Pv peptides some of which could be markers of hypnozoite carriage. Those participants who do and those who do not develop Pv relapses will be compared.

3 OVERVIEW OF STUDY DESIGN

3.1 PfSPZ Vaccine and PfSPZ-CVac

- 1) This is a double-blind, randomized, placebo-controlled, Phase 2 clinical trial.
- 2) 372 healthy Indonesian soldiers aged 18-55 years to be deployed to malarious eastern Indonesia will be randomized to immunization with three doses of PfSPZ Vaccine (Group 1), normal saline placebo (Group 2), PfSPZ-CVac (PfSPZ Challenge + CQ) (Group 3) or normal saline placebo + CQ (Group 4); randomization to the four groups will be 1 : 0.5 : 1 : 0.5.
- 3) The study has three phases: immunization and follow-up at the home base; deployment to eastern Indonesia for 6 to 9 months (surveillance period #1); redeployment to the home base for 6 months (surveillance period #2); study participation will be up to 20 months per participant, and the entire clinical trial will last approximately 28 months if deployment lasts 9 months.
- 4) A research monitor (RM) (= medical monitor = safety monitor) and a safety monitoring committee (SMC) will provide safety oversight.
- 5) External study monitoring will be the responsibility of Sanaria or Sanaria's designated and authorized representative in Indonesia.
- 6) Screening will be done within 56 days of enrollment and immunizations will be completed prior to deployment. Screening evaluation includes an electrocardiogram (ECG) performed at screening. Participants with clinically significant abnormal cardiovascular symptoms or findings will be excluded and referred to a cardiologist for further evaluation; individuals with a history of non-febrile seizures will also be excluded.
- 7) Solicited adverse events will be monitored for 7 days after each PfSPZ Vaccine/placebo administration and for 14 days after each PfSPZ-CVac/placebo administration; unsolicited adverse events will be followed during the immunization period and up to 2 weeks after the last immunization if the deployment schedule allows; serious adverse events (SAEs) will be monitored throughout the study. Follow-up of AEs occurs until resolution or stability.
- 8) Case report forms (CRFs) will serve as the repository of source documents and other relevant data for each study participant. Only information that cannot be collected initially into the CRF (namely, laboratory test results, ECGs and adverse event (AE) medical records, etc.) will first be collected onto separate source documents before transcription into the CRF. The information in the CRF will then be manually entered directly into the internet data system by independent data entry technicians.
- 9) Clinical and laboratory evaluations will occur at sites established at the battalion's home base and near the deployment area in eastern Indonesia.
- 10) Direct observation will be for 30 minutes following each dose of PfSPZ Vaccine or PfSPZ Challenge. For the 2nd and 3rd immunizations, allowable windows are assigned (see **Table 7**).
- 11) Both active and passive surveillance for clinical malaria cases will occur during surveillance periods 1 and 2; active surveillance will consist of questioning participants about malaria-related symptoms and assessing body temperature for presence of fever every two weeks and collecting thick and thin blood smears or rapid diagnostic tests (see below) from anyone with symptoms or signs of malaria; passive surveillance will be done by encouraging participants who feel ill to report to the

study clinic. First Pf clinical malaria cases are the primary vaccine efficacy (VE) outcome of the trial. First Pv clinical malaria cases are an important secondary outcome.

- 12) Thick and thin blood smears will be collected from asymptomatic individuals every 4 weeks and read retrospectively to identify all Pf and Pv new infections (important secondary endpoints).
- 13) During deployment, study participants will be assigned to duty posts without regard to study participation status, as treatment assignment will be blinded. Some of those posts are not directly accessible from the research team base in the field (remote posts). Study participants assigned to remote posts will be accompanied by a paramedic assigned to the research team. Those participants are designated by the terms “remote” vs. “accessible” because their management differs in terms of sample collection and safety precautions.
- 14) For accessible participants with symptoms consistent with malaria, thick and thin blood smears will generally be read within four hours. For remote participants with symptoms consistent with malaria, thick and thin blood smears will be prepared and stained within 24 hours and stored for analysis once there is access to microscopy. A rapid diagnostic test (RDT) for malaria will be performed immediately and the results will guide treatment. In both accessible and remote participants, a sample for qPCR will be taken before initiation of treatment. In accessible participants, a 5mL sample of venous blood for on-site purification of parasite DNA for later genomic analyses of the parasites will be taken before initiation of treatment.
- 15) Those positive and symptomatic for one species of parasite (i.e., Pf or Pv) will be treated for malaria with a first-line drug approved and recommended for use in Indonesia (this is DHA-PP for either species) and after a 28-day drug washout period (not at risk of malaria) will continue follow-up for clinical cases caused by the other species of parasite or for additional cases of clinical malaria caused by the first species of parasite. Participants experiencing first events with both Pf and Pv in eastern Indonesia will have reached the primary endpoint, a first clinical case of Pf, and an important secondary endpoint, first clinical case with Pv. They will begin contributing person-time at risk of relapse by Pv after departure for and then six months after arrival in the malaria-free home base. The total number of malaria cases will be an exploratory endpoint of the trial.
- 16) The first unblinded analysis will be conducted for safety endpoints and efficacy against first infection with Pf and Pv shortly after the battalion returns to home base. The primary analysis will be prepared at this time along with a Study Report. The research team conducting post-exposure surveillance will remain blinded as to vaccine or placebo assignment of the participants.
- 17) Additional analyses will be conducted after the participants have completed the 24-week follow up after returning from Eastern Indonesia and used to update the Study Report with the results of the Pv relapse component and with the additional analyses performed, such as those performed after confirmation of endpoints. This will constitute the final Clinical Study Report.

3.2 PfSPZ Vaccine

Participants will be randomized in a 1 : 0.5 ratio to receive either PfSPZ Vaccine (N=124) or 0.9% sodium chloride (normal saline=NS) (N=62), by direct venous inoculation (DVI) on study days designated PfSPZ Vaccine Day 1 (D1), PfSPZ Vaccine Day 8 (D8), and PfSPZ Vaccine Day 29 (D29). The dose will be 9×10^5 PfSPZ of PfSPZ Vaccine per dose.

PfSPZ Vaccine surveillance for solicited local AEs will be done retrospectively (on the day of vaccination, all participants will be informed to look at the injection site for +2 days after each dose and at the next visit after vaccination, the study team will ask the participant if there are any complaints in the area around the injection site), for solicited systemic AEs will be done for +7 days after each dose and for unsolicited AEs for +14 days after each dose. SAEs will be recorded throughout the study.

3.3 PfSPZ-CVac (PfSPZ Challenge + CQ)

On PfSPZ-CVac Day -2 (D-2), which is two days before the first dose of PfSPZ Challenge, participants will be given a single loading oral dose of CQ (10 mg/kg CQ base). They will then receive weekly CQ (5 mg/kg CQ base) so that CQ is given for a total of 63 days (10 doses), with the last dose +5 days after the third injection with PfSPZ Challenge on Day 62 (D62). In case of delays in immunizations, CQ administration will continue with additional weekly doses so that the last dose is no less than +5 days after the third injection.

Participants will be randomized in a 1 : 0.5 ratio to receive either PfSPZ Challenge or NS by DVI on PfSPZ-CVac Day 1 (D1), PfSPZ-CVac Day 29 (D29), and PfSPZ-CVac Day 57 (D57).

The dose will be 2×10^5 PfSPZ of PfSPZ Challenge per dose.

PfSPZ-CVac surveillance for solicited local AEs will be done retrospectively (on the day of vaccination, all participants will be informed to look at the injection site for +2 days after each dose and at the next visit after vaccination, the study team will ask the participant if there are any complaints in the area around the injection site), for solicited systemic AEs will be done continuously from Day -2 to Day 69 (+7 days after the last dose of CQ) and for unsolicited AEs from Day -2 to Day 71 (+14 days after the last dose of PfSPZ Challenge). SAEs will be recorded throughout the study.

3.4 Site Description:

Participants for this study will be drawn from the population of healthy adult soldiers aged 18-55 years about to deploy to eastern Indonesia. There will be several study locations, each with sub-sites where work will occur. The months for each activity have been projected based on an updated deployment schedule.

- Jakarta: months 0-18
 - OUCRU ID/PRBME-BRIN
 - IOCRL/FKUI
- Home base pre-exposure: months 0-4
 - Army base clinical study center
 - Off-base clinical vaccination site
- Travel to Eastern Indonesia by boat: est. month 4
- Eastern Indonesia exposure: est. months 4-13
 - Primary field study center
 - Remote study sites (approx. 9 months)
 - Hospital-based laboratory
- Travel to home base by boat: est. month 13

- Home base post-exposure: months 13-20
 - Army base clinical study center

3.5 Availability of Medical and Preventive Care:

Upon recruitment, study participants will have access to routine and acute care by the research team and its Indonesian Army medical component partners 24 hours a day, 7 days a week until the end of study participation. The provisions for this access will be complemented by increased levels of monitoring and surveillance while the participants are at high risk of naturally acquired malaria. Accessible participants will be within several hours or less traveling time to the clinical services offered by the research team at the primary field study center. Remote participants will be monitored 24/7 by an on-site paramedic trained, certified, equipped and supplied, and supervised by the Site Medical Officer posted at the primary field study center. All soldiers, regardless of study participation status will have access to these services. Any diagnosis of malaria is immediately followed by effective treatment of the acute attack with recommended chemotherapy (generally DHA-PP). In the instance of a diagnosis of Pv malaria DHA-PP, rather than CQ, is administered per national treatment guidelines due to resistance to CQ.

In accordance with the Indonesian Army standard of care, soldiers in the field diagnosed with malaria will be thus treated for the acute attack.

The Indonesian Army provides insecticide treated nets and access to diagnosis and treatment to mitigate the risk of malaria to its deployed soldiers. Their medical doctrine does not provide for the administration of chemoprophylaxis against malaria. The study team will encourage soldiers to use the malaria personal protection supplies and measures offered to them by routine Indonesian Army practices but will not take measures specifically intended to ensure their availability or adherence to their use. Participants in the study will have agreed to consume no antimalarial drugs for any reason, except those prescribed by the research team. The research team recognizes and acknowledges its reliance upon its own monitoring, surveillance, and diagnosis and treatment services to mitigate the risks of acute malaria - the primary risk to participants and non-participants equally and alike, and it is the primary responsibility of the research team to not permit any case of malaria in any soldier to progress to complications.

3.6 Participant Inclusion Criteria

1. A male aged 18-55 years at the time of screening.
2. Assigned to the battalion of study and programmed to accompany it to eastern Indonesia for the duration of the deployment.
3. Freely provides written informed consent to participate in the study.
4. Agrees to adhere to Indonesian military medical guidance regarding screening and treatment of malaria.
5. Physical examination and laboratory results without findings that would jeopardize the safety of the participant or the integrity of the study, and a body mass index (BMI) $\leq 35 \text{ kg/m}^2$.

3.7 Participant Exclusion Criteria:

1. Previous vaccination with an investigational malaria vaccine.
2. Use of an investigational or non-registered drug or vaccine other than the study vaccine(s) within 30 days before the first study vaccination, or planned use up to 30 days after last vaccination.
3. Chronic administration (defined as more than 14 days) of immunosuppressant or other immune-modifying drugs within six months before the first vaccination. This includes any dose level of oral steroids, but not inhaled steroids or topical steroids.
4. Administration or planned administration of 1 live or 3 or more other type vaccines in the period beginning 28 days before the first study vaccination and ending 28 days after the last vaccination.
5. Confirmed or suspected immunosuppressive or immunodeficient condition.
6. Confirmed or suspected autoimmune disease.
7. History of allergic reactions or anaphylaxis to CQ or other 4-aminoquinolone derivatives.
8. History of serious allergic reactions to a drug (anaphylaxis, or requiring hospitalization).
9. History of allergy to phosphate buffered saline or human serum albumin.
10. Use or planned use of any drug with anti-malarial activity during the course of the study except for antimalarial medication administered by study clinicians.
11. History of splenectomy.
12. Laboratory evidence of liver disease (the final decision will be made by the PI and clinical officers, but in general a participant will be excluded if any of the screening liver function tests (ALT, bilirubin, gamma GTP) are > double the upper limit of normal measured twice without an explanation for the abnormal values).
13. Laboratory evidence of renal disease (serum creatinine > 1.5 mg/dL, measured twice)
14. Laboratory evidence of hematologic disease (platelet count or hemoglobin <80% of the lower limit of normal for Indonesia, measured twice).
15. Abnormal screening ECG showing prolonged QTc interval (>450 msec) or any signs of arrhythmia/irregularity, ischemia, cardiac enlargement considered indicative of acute or chronic cardiovascular disease.
16. Acute or chronic pulmonary, cardiovascular, hepatic, renal or neurological condition, severe malnutrition, or any other clinical findings that may increase the risk of participating in the study as determined by the principal investigator or her designee.
17. Administration of immunoglobulin and/or any blood products within the three months preceding the first study vaccination or planned administration during the study period.
18. Simultaneous participation in any other interventional clinical trial.
19. Other conditions that in the opinion of the principal investigator or her designee would jeopardize the safety or rights of a participant in the trial or would render the participant unable to comply with the protocol or might compromise the integrity of the data.
20. Any evidence of active malaria, whether symptomatic or asymptomatic, confirmed by RDT, microscopy or PCR before first injection of PfSPZ Vaccine or PfSPZ-CVac, unless treated by the clinical team.
21. History of non-febrile seizures or atypical febrile seizures.
22. Under treatment for tuberculosis.
23. Laboratory evidence of active infection with hepatitis B or hepatitis C.

24. Participants with $\geq 10\%$ 5-year cardiovascular risk (fatal and non-fatal) based on the Gaziano scoring system (**Appendix A**); participants in the 18–34-year-old age group will be assessed as though they are in the 35–44 age group.
25. History of psychiatric disorders (such as personality disorders, anxiety disorders, or schizophrenia) or behavioral tendencies (including active alcohol or drug abuse) discovered during the screening process that in the opinion of the investigator would make compliance with the protocol difficult.

3.8 Treatment Assignment Procedures

3.8.1 Randomization procedures

Three hundred and seventy-two individual participants will be randomized to receive either PfSPZ Vaccine (N=124), NS placebo (N=62), PfSPZ-CVac (PfSPZ Challenge + CQ) (N=124), or NS placebo + CQ (N=62) (approximate numbers). Randomization/group assignment will be done with support from the data management company, StatPlus.

Each participant enrolled into the trial will be assigned a treatment code. The study site will be provided with a treatment code list to be kept in a secure place with access permitted only to the unblinded pharmacist and syringe preparation team. Participants who receive the first vaccination will not be replaced. Participants who withdraw before the first vaccination may be replaced with newly randomized participants. The reason for any unblinding will be documented, as well as any steps taken to prevent further such unblinding.

3.8.2 Masking procedures

Measures will be taken to keep participants, clinical investigators and all other staff involved in measuring study outcomes blinded to treatment allocation. Masking procedures are described in the SOPs for randomization, vaccine preparation and administration. PfSPZ Vaccine and PfSPZ Challenge have a colorless appearance and are administered by DVI using a needle and syringe. The saline placebo will appear as a colorless clear liquid of the same volume and administered in an identical-appearing syringe to the vaccine. The two products cannot be distinguished visually, by odor or consistency. Syringes will be prepared behind closed doors and labeled with the participant's identification number, then delivered to the vaccinator. This procedure will ensure that neither the participant nor the vaccinator will know if vaccine or placebo is given. The vaccine preparation and dilution staff will include the unblinded study site pharmacists with experience and training in PfSPZ Vaccine and PfSPZ Challenge preparation, and their role in the trial will be restricted to vaccine handling and preparation, with no role in post-vaccination assessments or follow-up of study participants.

The statistician at StatPlus will generate the randomization list. Access to unsealed copies of the randomization list will be limited to the pharmacist, the vaccine preparers and the StatPlus statistician. These individuals will be unblinded and will not be involved in study participants' further evaluation.

3.8.3 Reasons for delay or discontinuation of vaccination

The following criteria will be checked before each vaccination. If any become applicable before completion of vaccinations, further vaccinations will be postponed or not administered and the

participant will be followed for the duration of the study. If immunization is discontinued or immunizations are delayed by more than 1 week (delayed by more than 1 day for the second PfSPZ Vaccine administration), the participant will not be included in the per protocol analysis.

Use of any investigational drug or vaccine other than the study vaccine(s) during the immunization period.

- Use of a drug or vaccine during the study with antimalarial properties that in the investigator's or Sponsor's judgment might affect vaccine take or malaria risk.
- Chronic administration (defined as more than 14 days) of any dose level of immunosuppressant or other immune modifying drugs during the study period and chronic daily use of inhaled and topical steroids. Intermittent use of inhaled and topical steroids is allowed.
- Administration of live vaccines, immunoglobulin or blood products during the period starting from 28 days before the first study vaccination and ending 28 days after the last vaccination.
- Administration of 3 or more non-live vaccines not foreseen by the study protocol during the period starting from 28 days before the first study vaccination and ending 28 days after the last vaccination.
- Unresolved laboratory abnormalities that are deemed by the PI and/or the RM to be clinically significant and meriting delay or discontinuation. Transient laboratory abnormalities that have been documented to have returned to normal range before vaccination or which are not deemed clinically significant may not necessitate withdrawal, depending on the judgment of the PI in consultation with the RM.
- Acute disease at the time of vaccination, defined as the presence of a moderate or severe illness with or without fever. Vaccinations can be administered, at the investigator's discretion, to persons with a minor illness such as mild diarrhea or mild upper respiratory infection with or without low grade fever, i.e., axillary temperature $< 37.5^{\circ}\text{C}$. If there is acute disease and the vaccination is delayed, the participant may be vaccinated at a later date or withdrawn at the discretion of the investigator.
- Axillary temperature $\geq 37.5^{\circ}\text{C}$.
- Any additional reason deemed appropriate by the clinical investigator.

3.8.4 Reasons for discontinuation of chloroquine

CQ administration will be discontinued for individual participants if concerning signs or symptoms develop, including encephalopathy, anxiety, psychosis, weakness, significant disturbances of vision, retinal changes, hearing loss, hemolysis, renal dysfunction, bone marrow toxicity, skin eruptions, cardiotoxicity, allergic reaction, etc. If CQ is stopped, further immunizations with PfSPZ Challenge or placebo will also be stopped. Stopping the CQ in an individual will not lead to pausing the study or halting CQ administration in other participants unless there is an SAE deemed possibly, probably or definitely related to CQ that is unexpected and that results in such a recommendation by the SMC, a recommendation then instituted by the Sponsor.

3.8.5 Handling of withdrawals

Every effort will be made to assure the safety of any participant discontinued from receipt of additional vaccinations because of an AE or SAE or any other reason by continuing the safety follow-up procedures and data collection. The clinical team will provide appropriate, individualized care under medical supervision until the symptoms of any AE resolve or the participant's condition becomes stable. If withdrawal occurs at a time when a participant would potentially develop malaria from the PfSPZ Challenge product (within 14 days after administration), terminal treatment with a treatment course of antimalarial therapy will be given. Terminal treatment should be started no earlier than +7 days after injection of PfSPZ Challenge. If possible, participants who leave the study area will be traced and visited by clinical investigators to collect safety follow-up data.

Any participant who receives at least one vaccination and then is withdrawn from further vaccinations or misses vaccinations, who does not withdraw consent from participation in the trial, will be followed for incident malaria infections during the deployment and post-deployment periods.

3.8.6 Pausing or termination of study

The trial may be paused by Sanaria or the PI due to development of serious toxicity as indicated by laboratory or clinical assessments, or other major safety concerns. The trial may also be paused by the ECs/IRBs (Ethics Committee of the Faculty of Medicine, University of Indonesia and Oxford Tropical Research Ethics Committee, University of Oxford). or regulatory agencies (FDA, BPOM) if deemed necessary, and may be recommended for pausing or termination by the SMC. Refer to section on Pausing Rules and Safety Hold (see below) for more precise criteria. The trial may be terminated by Sanaria, the IRBs or regulatory agencies.

4 STUDY INTERVENTION INVESTIGATIONAL PRODUCT

4.1 Study Product Description

PfSPZ Vaccine: Sanaria Inc. (Sanaria) has developed a method to produce aseptic, purified, vialled, cryopreserved PfSPZ from aseptic *A. stephensi* mosquitoes infected with Pf. The PfSPZ of PfSPZ Vaccine are from the NF54 strain of Pf, which is thought to have originated in West Africa. The parasites are attenuated by exposure to irradiation [21, 55]. PfSPZ Vaccine is provided to the clinical team diluted in Diluent (phosphate buffered saline (PBS) with 1% human serum albumin (HSA)).

PfSPZ Challenge: The PfSPZ of PfSPZ Challenge are identical to those of PfSPZ Vaccine, but are not attenuated by exposure to irradiation, and thus are fully infectious [46, 56]. PfSPZ Challenge is provided to the clinical team diluted in Diluent.

Placebo: 0.9% sodium chloride used for injection in clinical settings (e.g. hospitals) will be used for the placebo.

Chloroquine (CQ): CQ phosphate is a 4-aminoquinoline, antimalarial agent for oral administration. It is highly active against the Pf (NF54) used to produce PfSPZ.

4.2 Acquisition

PfSPZ Vaccine, PfSPZ Challenge, Diluent, and placebo will be provided by Sanaria. Sanaria will ship these to Indonesia using established carriers as has been done for cited studies in the US [9-12, 21, 42], the Netherlands [40, 56], Germany [13, 46], Spain [45], UK [43], Tanzania [41], Kenya [44], Mali [16], and Equatorial Guinea [51], and ongoing studies in these countries and Burkina Faso.

Sanaria will ship PfSPZ Vaccine and PfSPZ Challenge cryopreserved in liquid nitrogen vapor phase (LNVP) in dry shippers from its manufacturing facility in Rockville, Maryland to Jakarta. It will also ship the Diluent and placebo, which are both shipped and stored at controlled room temperature (15-30°C).

4.3 Formulation, Packaging and Labeling

4.3.1 PfSPZ Vaccine and PfSPZ Challenge

PfSPZ Vaccine is supplied in vials containing 9.0×10^5 PfSPZ of PfSPZ Vaccine in a volume of 20 uL.

PfSPZ Challenge is supplied in vials containing 1.0×10^5 PfSPZ or 2.0×10^5 of PfSPZ Challenge in 20 uL.

4.3.2 Diluent

The diluent for PfSPZ Vaccine and PfSPZ Challenge is composed of 1% HSA in PBS. PBS is manufactured by Sanaria in compliance with good manufacturing practices (GMP). Sanaria purchases HSA (25%) approved for parenteral, IV administration to humans. After thawing, PfSPZ Vaccine and PfSPZ Challenge will be diluted in Diluent according to Sanaria's SOPs.

4.3.3 Sterile 0.9% sodium chloride placebo

The placebo is a 0.9% sodium chloride injection USP. It is a sterile, non-pyrogenic, isotonic solution of sodium chloride and water for injection and will be used as the placebo. Each mL contains sodium chloride 9 mg. It contains no bacteriostatic or antimicrobial agents, or added buffer. The 0.9% sodium chloride to be used will be approved for human use in clinical settings and will be obtained from Hospira Inc., a Pfizer company and an international supplier able to document GMP and a batch lot analysis to be kept in the study file. Sanaria will provide the aforementioned 0.9% sodium chloride to the clinical site. See [https://www.medline.com/product/Sodium-Chloride-For-Injection-10ml-by-Hospira/Saline/Z05-PF30165? 3](https://www.medline.com/product/Sodium-Chloride-For-Injection-10ml-by-Hospira/Saline/Z05-PF30165?_3).

4.3.4 Chloroquine

CQ phosphate is typically available as 300-310 mg CQ base. This study will obtain and use CQ from a domestic or international supplier able to provide a certificate of GMP and lot batch analysis for this drug to be kept in the study file. The likely source will be from Dublin PLC in the UK.

4.4 Product Storage

A chain of custody of all of the products to be administered to study participants will be maintained and documented. Access to those products will be limited to specifically authorized members of the pharmaceutical operations team or regulatory authorities or designated study monitors. They shall be stored at appropriate temperatures in secured-entry rooms also having secured-entry devices if appropriate.

Shipping will be performed in accordance with all FDA, U.S. Department of Transportation, Republic of Indonesia, and United Nations transport guidelines. Upon arrival in Indonesia, PfSPZ Vaccine and PfSPZ Challenge, which will be shipped in dry shippers, will be held in the same transport dry shippers and stored with appropriate security and monitoring. The storage temperature of the dry shippers will be constantly monitored using data loggers. Specifics of study product receipt, monitoring, release and site notification will be included in the protocol-specific SOP.

Unused PfSPZ Vaccine and PfSPZ Challenge cryovials retained in the dry shipper will be returned to Sanaria.

Diluent for PfSPZ Vaccine and PfSPZ Challenge and normal saline used for placebo will be stored at controlled room temperature (15-30°C). Details of storage and transfer of these products will be outlined in the protocol-specific SOP.

CQ tablets must be stored at controlled room temperature (15-30°C) and will be kept away from prolonged exposure to direct sunlight.

Any temperature chain deviation for the vaccine will be recorded and reported. Products shown exposed to temperatures beyond the tolerable range of those recommended shall not be used.

4.5 Dosage, Preparation and Administration of Study Investigational Product (IP)

Study vaccine will be prepared by unblinded vaccine preparation staff (Pharmaceutical Operations team) trained and certified by the Sponsor (Sanaria Inc.). PfSPZ Vaccine and PfSPZ Challenge will be thawed and diluted with the Diluent for dose administration. The dose will be prepared using an appropriately sized syringe and must be administered within 30 minutes of thawing. The pharmaceutical operations team will load vaccine or placebo according to randomized study number assignment. The placebo volume will be drawn using the same volume as the vaccine dose. Further details regarding dilution and syringe preparation will be included in Sanaria SOPs.

4.5.1 Administration of vaccine or placebo

Vaccine or placebo will be administered by blinded study staff given labeled syringes by the unblinded pharmaceutical operations team. Each dose of PfSPZ Vaccine, PfSPZ Challenge and NS placebo will be administered in about 0.5 mL by DVI into a vein in the participant's arm or hand using a needle and syringe according to SOP and the study schedule.

4.5.2 Dosing and administration of chloroquine

Loading Dose: 10 mg/kg of CQ base (will be orally administered as a single loading dose to the participant by the study staff via directly observed therapy on PfSPZ-CVac Day -2.

Weekly Dose: Subsequent doses of maintenance dose CQ (5 mg/kg CQ base) will be given weekly as a single dose. Doses will be given in a single calendar day either all at once or individually within one hour under direct observation with consumption of at least 200 mL of liquid to ensure ingestion.

For both the Loading Dose and the Weekly Dose, participants will be observed for 30 minutes to ensure that drug is not vomited. If vomiting occurs during observation, dosing of the product consumed will be repeated one time for the Loading Dose and up to two additional times for the Weekly Dose.

Depending on the manufacturer and the precise mg content of the CQ tablets, adjustments to the doses specified above may be made, following the manufacturer's instructions.

Additional adjustments may be needed in CQ dosing if soldiers are sent for training exercises, for example. Weekly administration will be maintained as best as possible.

4.5.3 Modification of study investigational product (IP) for a participant

Dosing of PfSPZ Vaccine, PfSPZ Challenge, or CQ will be discontinued if found to be unacceptably toxic. If a participant experiences notable toxicity related to PfSPZ Vaccine, PfSPZ Challenge or CQ, then the investigators and the Sponsor, in consultation with the Research Monitor and the SMC will determine if subsequent vaccinations or administration of CQ in that participant is acceptable. If a participant experiences an SAE deemed to be related to PfSPZ Vaccine, PfSPZ Challenge, or CQ, they will not receive the relevant subsequent injections or doses of study IP unless so recommended by the SMC, Sponsor, and PI.

4.5.4 Accountability procedures for the study investigational product (IP)

The PI alone has the authority to distribute or dispose of the IP as instructed by Sanaria and has the responsibility for the accountability of it. That authority may be delegated to the Site Medical Officer while the PI is not on site. The PI may also delegate to the Site Research Pharmacist responsibility for IP accountability. The Site Research Pharmacist will be responsible for maintaining complete records and documentation of IP, accountability, dispensation, temperature monitoring, storage conditions, and final disposition of the study product according to Sanaria SOPs. All study IPs, whether administered or not, must be documented on the appropriate study IP accountability record or dispensing log. All and any unused IP will be retained, either shipped back to Sanaria.

Upon completion of the study and after the final monitoring visit, any remaining unused study IP will either be returned to Sanaria.

4.6 Concomitant Medications/Treatments

At each study visit/contact, investigators will question the participant about any medication taken, including traditional, herbal, supplements and over-the-counter medicines. Concomitant medication, including any vaccine other than the study vaccines, including any specifically contraindicated or administered during the period starting from -28 days before study start and ending at the end of the study follow-up period will be recorded with trade name and/or generic name of the medication, medical indication, start and end dates of treatment.

Medications that are not permitted during the follow-up period, unless prescribed by the research medical team, include, but are not limited to: CQ, sulfadoxine-pyrimethamine, trimethoprim-sulfamethoxazole, azithromycin, amodiaquine, artemether-lumefantrine, DHA-PP combination therapies, halofantrine, quinine, doxycycline, mefloquine, primaquine, tafenoquine, atovaquone/proguanil or other drugs with known antimalarial properties. These medications will be prescribed by study clinicians when indicated and adjustments will be made as needed for analyses of time period under risk for malaria infection in the statistical analysis plan.

5 STUDY SCHEDULE

5.1 Recruitment and Screening

Prior to active screening, the research team will engage study battalion commanders and soldiers in order to familiarize both with the aims and procedures of this trial. Moreover, the vital importance of voluntary consent and freedom from coercion to participate or ostracism for not participating will be strongly emphasized. The team acknowledges the intrinsically coercive character of military culture and proactively mitigates these risks to study participants and the trial. The chain of command above the study battalion issues orders to its commanders to not issue a direct order to participate or to punish those who decline consent to participate or who choose to withdraw from the study. In two prior clinical trials employing Indonesian Army battalions (2011, 2014), a total of 362 eligible soldiers were invited to enroll and 37 declined consent, and another 6 withdrew from participation [57, 58].

Recruitment will progress until 372 adult males who fulfill the eligibility criteria are enrolled. If there are difficulties reaching this number, a lower number such as 340 participants will be deemed acceptable if agreed to by the sponsor and responsible investigator. After community information is provided as described above, all interested, potentially eligible participants will be invited to visit the study clinic on a specific date and time. The individual consent process will be conducted in private areas to ensure confidentiality, to reduce the likelihood of other participants influencing their decision, and to allow further time to make a final decision. A single informed consent document will be used for screening and other study procedures. After the study has been explained to individual participants they will be provided with a copy of the consent form. The form is written in Indonesian language and read to or provided to the participant in that language. The participant is asked explicitly if he has understood this form or has any questions regarding it. He is invited to sign the form in the presence of a disinterested witness who also signs the form. He may leave and return later with his decision, allowing time to carefully consider involvement in the study and ask any questions that he may have.

All screening tests, medical history and physical and laboratory examinations will be performed only after study consent has been obtained. Screening visit procedures, including informed consent, eligibility review procedures (physical exam, laboratory test, etc.) and randomization may occur over several days. Study clinicians may manage uncomplicated acute illness without exclusion. In the event of more complicated or chronic conditions, such as renal or heart disease (and other conditions as listed in the exclusion criteria), participants will be excluded from further participation and referred to appropriate sources of medical care. A 12-lead ECG will be done and assessed by the examining study physician. QT_c interval elongation may prompt exclusion along with any other indication of cardiac pathologies (see exclusion criteria).

An eligibility checklist will be prepared for each participant as a case report form (CRF). A medical history will be taken with special attention to recurrent infections to suggest immune suppression, previous history of splenectomy and prior vaccine reactions. Concomitant medications and the history of vaccination against COVID-19 will be documented and a physical examination, vital signs, anthropometric measurements (height and weight) and laboratory screening test will be performed. Participants excluded from this study because of significant abnormalities will be managed initially by study clinicians and referred to the local military health center for evaluation as necessary. Laboratory

studies may be conducted at other times during the course of the trial if the investigators judge it necessary for the safety of the participant. Screening and follow-up diagnostic laboratory testing will be performed at the clinical laboratory at the study site and at a local reference laboratory if needed. Should a participant change his mind and decline to participate before vaccination, he may be replaced by another participant. Soldiers who withdraw after the first vaccination may not be replaced.

One of the screening tests will be the collection of blood for blood smears and for qPCR. Any participant with malaria parasitemia will be treated prior to immunization due to the immunosuppressive effects of parasitemia that inhibit the induction protective immunity [33-35] including by PfSPZ Vaccine (Diawara and Healy, unpublished). Blood smears will be read on site, and samples for qPCR will be transported as needed to perform this more sensitive assay. DHA-PP will be used for treatment in most cases. An interval of five days between the third dose of DHA-PP and first vaccination is prescribed.

5.2 Enrollment/Baseline and Subsequent Vaccination Visits

In this study, after a participant is found to be eligible for inclusion, the participant will be randomized to one of the four groups. Treatment allocation, prepared by the StatPlus biostatistician, will be communicated as a two-step process.

Step 1: Randomization to PfSPZ-CVac / corresponding placebo group, or to PfSPZ Vaccine / corresponding placebo group. This open-label randomization assignment will be revealed when the clinical team opens an individualized envelop that assigns the next eligible participant to one main group or the other (details provided in the randomization SOP). If randomization is to PfSPZ-CVac / corresponding placebo group, immunizations will begin promptly. If randomization is to PfSPZ Vaccine / corresponding placebo group, immunizations will be delayed approximately 1 – 2 months so that participants in the two main groups complete immunizations at about the same time.

Step 2: Randomization to vaccine or placebo. This double-blind randomization will be revealed only to the unblinded pharmacy team at the time of immunization.

PfSPZ Vaccine: Participants will be randomized to receive either PfSPZ Vaccine or NS and receive their first immunization on Day 1. Subsequent vaccinations will occur at Day 8 and Day 29.

PfSPZ-CVac: Participants will be randomized to received either PfSPZ-CVac (PfSPZ Challenge + chloroquine) or NS + chloroquine. They will receive a loading dose of CQ -2 days prior to their first injection with PfSPZ Challenge/NS. This will be Day -2. Immunization with PfSPZ Challenge or NS will be on Day 1. Subsequent vaccinations will occur at Day 29 and Day 57.

Both vaccines: Prior to each vaccination, criteria for continued eligibility will be reviewed and verified using an eligibility checklist and targeted physical exam with documentation of vital signs (axillary temperature, blood pressure, pulse, respiratory rate). Baseline venous blood will be collected for laboratory analysis before vaccination.

After the above procedures are completed and the participant will be vaccinated according to the vaccine administration SOP. The research medical team will always be present during vaccinations and will be supplied, equipped, and trained to cope with any unpredicted medical emergency, to include anaphylaxis and cardiac or respiratory arrest. The participant will be considered officially enrolled in the study once the first vaccination has been administered (replacement of participants who drop out is allowed up to the point of first vaccination, but not thereafter).

After each vaccination, participants will be observed for local and systemic reactions for a minimum of 30 minutes and their vaccination site(s) will be examined for any abnormalities. Information regarding signs and symptoms will be solicited from participants and recorded by the investigators, and vital signs including pulse, respiratory rate, blood pressure, and axillary temperature will be noted. At the end of the visit, participants will be instructed to return to the research clinic immediately should they manifest any signs or symptoms they perceive as concerning or serious.

Details of the schedule of events, including post-vaccination safety and immunogenicity laboratory analyses, are included in the Study Schedule and Procedures Table (**Table 7**).

Addendum added with Version 4.0 of the protocol: A shortage of funds for completing the trial as planned may result in the inability to collect most of the research samples slated for the post-redeployment period. In addition, the final set of safety laboratory testing may be omitted, as the scheduling of these laboratory tests six months after redeployment and more than a year from vaccination was primarily for research, not safety purposes. However, the first set of safety laboratory testing post-redeployment will be conducted (with an option to conduct the tests in Papua prior to redeployment rather than after returning to the home base in Bangkinang), as this is important from the perspective of participant safety. For example, in a prior study of a battalion redeploying back to its home base in Malang after deployment to Papua, 12% of soldiers had grade 1 or grade 2 anemia at the time of their return, likely as a result of exposure to malaria infections during the deployment.

PFSZ Vaccine Group	Screening Period	Vaccination Period (Pre-Exposure)								Follow-up Period #1 (Exposure) (approximately 6 - 9 months)																								Follow-up Period #2 (Post-Exposure)																																																																																																															
		(43 days)								(6 months)												(9 months)												(6 months)																																																																																																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100																																												
Study Weeks Relative to First Vaccination*	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100																																												
Study Days Relative to First Vaccination / return to Jawa*	-2	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	148	155	162	169	176	183	190	197	204	211	218	225	232	239	246	253	260	267	274	281	288	295	302	309	316	323	330	337	344	351	358	365	372	379	386	393	400	407	414	421	428	435	442	449	456	463	470	477	484	491	498	505	512	519	526	533	540	547	554	561	568	575	582	589	596	603	610	617	624	631	638	645	652	659	666	673	680	687	694	701	708	715	722	729	736	743	750	757	764	771	778	785	792	799	806	813	820	827	834	841	848	855	862	869	876	883	890	897	904	911	918	925	932	939	946	953	960	967	974	981	988	995	1002
Study Visit	SC	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30	V31	V32	V33	V34	V35	V36	V37	V38	V39	V40	V41	V42	V43	V44	V45	V46	V47	V48	V49	V50	V51	V52	V53	V54	V55	V56	V57	V58	V59	V60	V61	V62	V63	V64	V65	V66	V67	V68	V69	V70	V71	V72	V73	V74	V75	V76	V77	V78	V79	V80	V81	V82	V83	V84	V85	V86	V87	V88	V89	V90	V91	V92	V93	V94	V95	V96	V97	V98	V99	V100																																												
Visit Window*	-5d	0	1d	8d	15d	22d	29d	36d	43d	50d	57d	64d	71d	78d	85d	92d	99d	106d	113d	120d	127d	134d	141d	148d	155d	162d	169d	176d	183d	190d	197d	204d	211d	218d	225d	232d	239d	246d	253d	260d	267d	274d	281d	288d	295d	302d	309d	316d	323d	330d	337d	344d	351d	358d	365d	372d	379d	386d	393d	400d	407d	414d	421d	428d	435d	442d	449d	456d	463d	470d	477d	484d	491d	498d																																																																							

9= Post deployment hematology, chemistry, serology and PBMCs may be drawn prior to departure from Papua.

PFSPZ-CVAc Group	Screening Period	Vaccination Period (Pre-Exposure)											Follow-up Period #1 (Exposure) (approximately 0 - 9 months, +54 days)																				Follow-up Period #2 (Post-Exposure)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
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		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998

*Study Weeks and Days based on a two week travel period between Pekanbaru and Eastern Indonesia and the assumption a volunteer will complete the first visit following deployment from and return to immediately after arrival; during the Follow-up Period #1 (Exposure) and Follow-up Period #2 (Post Exposure) these Weeks and Days will likely be later than this estimate.

** if moved more than 2 days, may need to add, subtract or adjust CQ dosing.

***First Exposure Visit (FEV) and First Post-Exposure Visit (FPEV) will be conducted once volunteers arrive in Eastern Indonesia and Homebase, respectively, targeting within the first +28 days of arrival.

1= During vaccination days, vital signs will be collected twice, once prior to vaccination (to confirm continued eligibility), and once at least 30 minutes post vaccination.

2= Hematology and biochemistry= complete blood count (CBC), creatinine, ALT (GOT)

3= At screening only, biochemistry will also include bilirubin, gamma GTP and glucose.

4= For volunteers that are symptomatic and/or are positive for parasitemia, refer to Table 8 for additional procedures.

5= During the Exposure Period, at accessible, non-remote sites, a 0.5 mL sample for PCR will be collected; this will not be collected for volunteers in remote locations unless it is possible.

6= Solicited AE collection will be through +7 days post the last CQ administration on Day 69, but the collection will be performed on Day 71

7= The first (morning) doses each day will be administered by DOT, and study subjects will take doses later in the day on their own

8= During the vaccination period, windows are relative to previous vaccination; during Follow-up Period #1 (Exposure) and Follow-up Period #2 (Post Exposure), visit windows are relative to the first visit in each period.

9= CQ dosing maybe adjusted according to manufactures specifications.

10= After a volunteer completes Screening Visit 1 and screening results are reviewed, if found eligible the volunteer will be enrolled and randomized to one of four treatment groups (randomization will be revealed to the clinical team as a two-step process -- see text).

11= Optional

12= Post deployment hematology, chemistry, serology and PBMCs may be drawn prior to departure from Papua.

5.3 Chloroquine Chemoprophylaxis for PfSPZ-CVac Groups

Participants in the PfSPZ-CVac group (Group 3) or corresponding placebo group (Group 4) will receive CQ phosphate in a standard prophylactic regimen of a loading dose of CQ (10 mg/kg CQ base) two days before (Day -2) the first dose of PfSPZ Challenge or normal saline. Subsequent doses of maintenance dose CQ (5 mg/kg CQ base) will be given once a week, starting seven days later on Day 6, which is 5 days after PfSPZ Challenge or normal saline administration on Day 1, for a total duration of 9 weeks and 10 doses (**Table 7**). As stated above, dose may be adjusted slightly depending on the mg content of the tablets and to account for delays resulting from training periods away from the home base.

CQ chemoprophylaxis will be given under direct observation (DOT) by a study physician or delegate whenever possible. If a participant vomits within 30 minutes the dose will be repeated; if they vomit more than twice for a given CQ dose, they will be withdrawn from the study unless they already received PfSPZ Challenge. If so, they will be permanently halted from receiving additional PfSPZ Challenge, but will need to continue with safety follow up due to risk of onset of acute malaria. The follow-up plan will be individualized by a study physician.

An oral examination can be considered after CQ administration to make sure that the tablet(s) have been swallowed, if acceptable to the research participant and physician.

In case of study halt or delayed administration in a given participant, CQ administration may be continued weekly until a decision is made to resume vaccination.

5.4 DHA-PP

DHA-PP will be the first line drug for treatment of Pf, Pv or other species of malaria. It is administered over three consecutive days for a total of three doses taken at approximately the same time each day. Participants with body weight 36 to <60 kg will receive 3 daily doses of 3 tablets (each tablet 320 mg DHA / 40 mg PP), those with body weight 60 to <80 kg will receive 3 daily doses of 4 tablets and those with body weight 80 kg or greater will receive 3 daily doses of 5 tablets.

DHA-PP will be administered with a snack. High-fat meals in proximity to DHA-PP administration will be avoided, as they significantly accelerate the absorption of piperaquine, thereby increasing the risk for prolongation of the QTc interval. Normal meals do not alter the absorption of piperaquine.

DHA-PP administration will be administered orally by direct observation (DOT). An oral examination can be considered after DHA-PP administration to make sure that the tablet(s) have been swallowed, if acceptable to the research participant and physician.

After each of the three doses, treated individuals will be observed for 30 minutes for vomiting. If a tablet is vomited within 30 minutes of administration, it may be re-dosed. If vomiting occurs again, a clinical consultation will be performed to determine the best course of action.

DHA-PP will be administered to any participants found to be positive for malaria by blood smear or PCR prior to the first dose of vaccine or placebo. Treatment will be administered, and vaccine/placebo postponed until a minimum of five days after the third dose of DHA-PP.

5.5 Vaccination Follow-up

PfSPZ Vaccine Group:

PfSPZ Vaccine is administered on Days 1, 8, and 29. Participants will be followed for + 2 days after each dose of PfSPZ Vaccine for solicited local AEs, for +7 days after each dose of PfSPZ Vaccine for solicited systemic AEs, and for +14 days after each dose of PfSPZ Vaccine for unsolicited AEs. This will be done by seeing them during clinic visits and inquiring retrospectively regarding AEs since the prior clinic visit. SAEs will be recorded throughout the study.

PfSPZ-CVac Group:

PfSPZ Challenge is administered on Days 1, 29, and 57. However, participants will also be receiving CQ as outlined in **Table 7** (above). Participants will be followed for +2 days after each dose of PfSPZ Challenge for solicited local AEs, and continuously from Day -2 to Day 69 (+7 days after the last dose of CQ) for solicited systemic AEs and from Day -2 to Day 71 (+14 days after the last dose of PfSPZ Challenge) for unsolicited AEs. This will be done by seeing them during clinic visits and inquiring retrospectively regarding AEs since the prior clinic visit. SAEs will be recorded throughout the study.

Details of the schedule of events, including laboratory analyses, are included in the Summary of Study Procedures (**Table 7**). A targeted physical examination is an optional component of the evaluation by the physician clearing a participant for immunization, depending on his or her clinical judgement.

Presumptive treatment with antipyretics/analgesics or anti-inflammatory drugs on +7 and +8 days after the first injection of PfSPZ Challenge (Days 8 and 9):

As discussed earlier in the Background section describing clinical trials with PfSPZ-CVac, some of the malaria-naïve individuals receiving higher doses of PfSPZ Challenge (e.g. the 2×10^5 PfSPZ planned for this clinical trial) under CQ cover will likely experience symptoms of malaria (fever, chills, headache, etc.) during the brief period of transitory parasitemia that follows +7 to +9 days after administration. These adverse events, which may attain grade 3 in severity, predictably begin on the evening of the seventh day following PfSPZ Challenge administration (Day 8 in the current study) and almost always resolve within 24 hours of onset. When the dosing schedule for PfSPZ Challenge is 0, +4, and +8 weeks, as in the current study, the most severe signs and symptoms tend to occur after the first dose. Signs and symptoms ameliorate with subsequent doses due to the rapid development of immunity. In the 17-I-0067 trial in malaria naïve Americans by the LMIV at the NIH Clinical Center, where the dose of PfSPZ Challenge was also 2×10^5 PfSPZ, presumptive treatment with antipyretics/analgesics/anti-inflammatory drugs starting on the morning of the seventh day prevented the occurrence of grade 2 or grade 3 signs or symptoms of parasitemia; as a result, only 2 of 5 participants experienced transient grade 1 symptoms, and 3 were symptom-free. The study participants were thus minimally inconvenienced by the transient parasitemia, and were able to pursue their normal daily activities. Because many of the study participants in the Indonesian trial will be malaria-naïve and have daily military duties to perform, the same procedure will be done.

The LMIV team successfully used ibuprofen 400 mg every 6 to 8 hours or naproxen 500 mg every 12 hours. These drugs, as well as acetaminophen / paracetamol or another equivalent over-the-counter drug, will be available to the clinical team, to allow a degree of flexibility regarding

which drug or drugs are used, depending upon experience and study participant tolerance. The presumptive treatment will be given as directly observed treatment (DOT) after the first vaccination with PfSPZ Challenge, when the study participants are first seen on Day 8 and Day 9, and additional pills will be provided to each participant that are sufficient for continued dosing the remainder of each day, evening and night, including instructions regarding the recommended time(s) for administration. If the study physicians find that presumptive treatment is not needed for a given study participant, or with second and third injections of PfSPZ Challenge, there is flexibility to adapt or discontinue regimens for individual study participants according to their needs. However, if needed, treatment may be administered on days +7 and +8 after the second and third immunizations (Days 36 and 37, 64 and 65).

Guidelines for additional treatment with an antimalarial:

As discussed earlier, there was one instance of likely surreptitious non-compliance with the ingestion of CQ in the TÜCHMI-002 trial in Germany; this study participant had sub-therapeutic levels of CQ in his blood, and required additional treatment with an antimalarial on the 13th day after injection of PfSPZ Challenge (Day 14). The fact that this did happen mandates close follow-up in the Indonesia trial, to rapidly identify any indication for additional treatment. To do this, participants assigned to the PfSPZ-CVac groups (Groups 3 and 4) will be seen on +7, +8, +9 and +12 or +14 days after each immunization with PfSPZ Challenge (+12 days following the first two immunizations and +14 days following the third). At these visits, thick and thin smears, blood blotted onto filter paper (60 ul), blood placed into a microtube (500 ul) for retrospective qPCR analysis, and blood blotted onto filter paper for genome sequencing will be collected. Blood smears will be read retrospectively (but generally within 48 hours) in asymptomatic research participants, and in real time (the same day, generally within 4 hours, if collected prior to 8:00 pm) *in any participant with signs or symptoms of malaria*. Any research participant whose blood smear remains positive will continue to have a blood smear checked daily until negative.

Consecutive blood smears that do not turn negative, or worsening signs and symptoms that raise clinical concern, or any combination of clinical factors considered by the on-site physician, may be used to justify re-treatment. The following will serve as flexible guidelines to be taken into consideration:

- High parasite density (parasitemia >1000 parasites/ μ L \approx 0.025% parasitemia) at any point.
- Sustained parasitemia (>250 parasites/ μ L) for 3 days.
- The detection of parasitemia on Day 13, 41 or 71.

5.6 Exposure Follow-up

After the follow up visit +14 days after the third dose of PfSPZ Vaccine and +14 days after the third dose of PfSPZ-CVac, participants will be considered fully processed and ready for arrival in malarious eastern Indonesia. The one exception will be if retrospective PCR reveals that any of the participants were positive for malaria parasitemia prior to vaccination, indicating that malaria may have been acquired naturally either at the army base or while the soldier was traveling or previously deployed. If this is the case, if any such soldier was not already identified and treated prior to vaccination, he will be treated either prior to deployment, during transport to the deployment site, and/or after arrival at the deployment site, so that he will be parasite-free at the start of surveillance.

On visit +14 days after the third dose of vaccine or placebo (or another day prior to departure), the participants will have a baseline collection of samples for parasitology assessment, including thick and thin smear, blood blotted onto filter paper (60 µl) and placed into a microtube (500 ul) for retrospective PCR analysis, and samples for genome sequencing (in this case both 60 ul blood blotted onto filter paper and 5 mL placed into a tube) (**Table 7**).

Arrival in eastern Indonesia will mark the beginning of the period at risk of naturally acquired malaria and close monitoring and supervision. As soon as it is feasible after arrival, all participants will have a repeat thick and thin blood smear and qPCR sample collection (60 ul blood blotted onto filter paper and 500 ul into a microtube), blood blotted onto filter paper for genome sequencing (filter paper only, no 5 mL sample) and an 8-10 mL (target, 10 mL) blood sample for serology. Vital sign measurement, and as necessary, a focused physical examination, may be performed. If it is logistically preferable to obtain the serology sample at a separate time from the other blood samples, that is permitted.

The thick and thin blood smears and qPCR samples will confirm that no study participant has acquired malaria at the time of departure from Sumatra or while traveling to Papua. The additional serology is required to establish a uniform pre-exposure baseline for all participants. Selecting a timepoint after arrival in Papua will adjust for any unanticipated differences between the PfSPZ Vaccine / placebo and PfSPZ-CVac / placebo groups in the time interval between the third immunization and arrival in Papua. It will also more accurately reflect the maturation of the immune response after the third vaccine dose than samples taken earlier than fourteen days after the third dose prior to deployment, in case the deployment date does not allow a 14 day wait period before obtaining the serology sample. The serological data will be used to assess correlates of protection, an important study objective.

Assessments will continue roughly every two weeks through the entire period that the soldiers are in eastern Indonesia, while the ongoing collection of samples will occur automatically every four weeks. Central to these assessments is asking about the occurrence of symptoms possibly related to malaria infection, and the measurement of body temperature for evidence of fever. The list of symptoms to be solicited can be found in section 7.7. In addition to this scheduled active case detection for clinical malaria, if a participant complains of illness, he will be encouraged to report day or night to the study clinic in an unscheduled visit. On evaluation by the clinician, if the presentation is suggestive of acute malaria, thick and thin blood smears, the two qPCR samples, and blood blotted onto filter paper for genome sequencing will be taken; vital signs will be recorded and a physical examination appropriate for the symptoms will be conducted. If the presentation is not suggestive of acute malaria, an alternative, clinically appropriate assessment will be made.

If positive for malaria, a number of procedures are initiated (**Table 8**), including immediate initiation of treatment with DHA-PP to clear the blood of parasites and to resolve acute illness. For symptomatic participants, the treatment should be provided within 6 hours of the time that the thick blood smear is read. It will be considered a major deviation should the treatment for symptomatic participants be delayed over 48 hours. The thick blood smears for asymptomatic participants may not be read for months after they are taken. However, once a positive result is obtained, the participant should be evaluated, and if treatment is indicated, treatment should be provided within 48 hours. It will be considered a major deviation should the treatment for asymptomatic participants be delayed over 4 days.

Primaquine therapy against latent hepatic stages of *P. vivax* or *P. ovale* will not be administered during this exposure period. The case for doing so may be summarized as follows:

Participant safety:

- 1) Participants shall be under close monitoring for signs of active symptomatic malaria, and those participants shall be promptly treated with therapy that will arrest that acute attack and restore the health of the participant.
- 2) The primaquine therapy prescribed under national treatment guidelines, 0.25mg/kg/day x14d is now known to be approximately only 50% efficacious with supervised drug administration (Sutanto et al. ms in preparation) in this very population, i.e., soldiers infected by *P. vivax* in northeastern Papua. We also know that unsupervised adherence to the 14 day regimen is quite poor. A large study of unsupervised primaquine therapy in Papua measured less than 10% effectiveness [<https://pubmed.ncbi.nlm.nih.gov/28850568/>].
- 3) The very high risk of reinfection by mosquito bite greatly diminishes the benefit of radical cure in this population, which is realized only in the weeks and months following therapy in the absence of reinfection.
- 4) We assert that participant safety under close supervision and prompt diagnosis and treatment of any acute malaria, and being highly alert to that, will exceed that of patients given ineffective treatment without supervision and a potentially dangerous presumption of protection from subsequent relapses.

Technical necessity:

- 1) We have evidence from animal models [Scheller LF, Azad AF, PNAS USA 1995; 92: 4066-8] that suggest the administration of primaquine may interfere with the durability of vaccine induced protection involving living hepatic stage parasites. The unsupervised administration of primaquine may introduce uncontrolled variability directly impacting the primary and secondary endpoints of this trial.
- 2) The administration of primaquine would entirely prevent this trial from shedding any light on the potential ability of this vaccine to prevent the formation latent *P. vivax* hypnozoites. This may be one of the most important secondary endpoints of this trial, which, if proven true, would transform vaccination strategies regarding this otherwise difficult and dangerous to treat form of vivax malaria.

The ethical analysis by Cheah et al. [<https://pubmed.ncbi.nlm.nih.gov/29510711/>] concluded that the use of a primaquine placebo was both ethically sound and technically necessary in assessing the therapeutic efficacy of anti-relapse therapy for *P. vivax*. We are assessing the efficacy of two vaccines rather than that of primaquine, but the guiding principles are essentially the same. Soldiers diagnosed with *P. vivax* in Papua will later be treated with primaquine if indicated, just not while in Papua. That indication would be another diagnosis of *P. vivax* during the 24 weeks of post-return follow up. The absence of such would be considered consistent with freedom from hepatic latency and any need for primaquine therapy.

Venipuncture will be done to obtain a venous blood sample (5 mL) for more in-depth parasite genomic analysis than is possible with a filter paper sample. Venous blood will not be collected at the remote sites; instead, finger prick blood will be collected. In an asymptomatic participant, this will be used for thick and thin blood smear (to be read later as microscopy may not be available at remote sites) and for blotting onto filter paper x 2, one for retrospective PCR and one for genome sequencing. In a symptomatic participant, the same samples will be drawn

from a finger prick, and in addition 20 ul will be used for the conduct of a rapid diagnostic test (RDT) for malaria. The RDT will be performed immediately, and the results used to guide treatment. In all instances of acute malaria confirmed by microscopy (accessible) or RDT (remote), treatment will be administered immediately following blood collection.

Every effort will be made to ensure compliance with visits. If a participant does not appear for a scheduled clinic visit, a study site staff member will visit him and request permission to collect the requisite samples for examination at the laboratory. If an SAE has occurred, appropriate measures will be taken to notify the appropriate individuals and organizations as per standard procedures.

Routine blood smears from asymptomatic research participants will not be read in real time. However, blood smears from participants showing signs and symptoms of malaria will be read within 4 hours (except for participants in remote locations).

Table 8. Summary of Planned Clinical Procedures with Confirmed Malaria

	Accessible Participants							Remote Participants*						
	Day post diagnosis							Day post diagnosis						
Days of follow-up (D0 = diagnosis)	0	1	2	7	14	21	28	0	1	2	7	14	21	28
Visit window (+/- number of days)	NA	0	1	2	3	4	4	NA	0	1	2	3	4	4
Visit number	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Procedure:														
1 st microscopist reads blood smear as positive; 2 nd or 3 rd confirms diagnosis	x													
Paramedic obtains positive RDT								x						
Vital signs and medical history	x	x	x	x				x	x	x	x			
Focused physical exam	x													
Parasite genome sequencing 5 mL	x													
Parasite genome sequencing filter paper	x			x			x	x			x			x
Communicate findings to medical officer / receive treatment instructions								x						
Administration of prescribed treatment (usually DHA-PP)	x	x	x					x	x	x				
Blood smear exam**				x	x	x	x							
Blood smear stained and stored								x			x	(x)	(x)	x
qPCR filter paper	x			x			x	x			x			x
qPCR microtube	x			x			x							
Resumption of person-time at risk							x							x

(x) = if possible

* It is possible that soldiers will be sent on missions to remote areas during deployment, and that members of the OUCRU ID team will not be allowed to accompany the soldiers for security and safety reasons. If this circumstance arrives, the OUCRU ID will train the Army medics accompanying the soldiers on the mission to collect as many of the above samples as possible.

** If D7 TBS is negative and participant did not have symptoms, TBS from D14, D21, D28 do not need to be read unless symptomatic.

5.7 Post Exposure Follow-up

After ~6-9 months of deployment, participants will return to the duty station at their home base. If staffing and resources allow, participants will undergo a baseline parasitology assessment that is the same as the baseline assessment done prior to their departure to eastern Indonesia 6-9 months earlier (see above; includes 5 mL for in-depth genome sequencing).

Back at their base, the soldiers will be followed for approximately another 24 weeks. The primary means of follow-up will be passive case detection. In other words, soldiers will be instructed to report any illness to the OUCRU ID clinical team, and if such illness is reported, the OUCRU ID team will interview the soldier, obtaining vital signs, a medical history, a physical examination if indicated, and finger-prick blood for a TBS. If the TBS is positive, the soldier will be treated with DHA-PP per national guidelines, including administration of primaquine (0.5 mg/kg/day for 14 days) in the case of *P. vivax*, to prevent any further *P. vivax* relapses. Administration of these medications will not be supervised, and follow-up TBS will not be done unless there is persistence of clinical symptoms.

In addition to passive case detection, there potentially may be active case detection. This will be done by conducting visits to every soldier every 4 weeks (instead of every two weeks as was done during deployment), if staffing and resources are sufficient to conduct these visits. Each visit will include at a minimum active questioning regarding malaria symptoms and fever. If resources allow, there may be additionally the acquisition of a blood sample for blood smear, and if possible, blood blot on filter paper for qPCR, blood in a microtube for qPCR, blood blot on filter paper for genome sequencing. In addition vital sign measurement may be done, and it will be determined if the participant has had symptoms consistent with malaria during the past 4 weeks and if they received treatment for malaria. If determined necessary, focused physical exam may be done at the discretion of the study clinician. If the blood smear is positive and personnel and facilities are available, follow up will be as above for Exposure Follow-up (section 5.6), including the 5 mL sample for in-depth genome sequencing, but this is now optional given the resource restrictions imposed on the study.

Follow-up of soldiers found to have malaria will include at minimum provision of DHA-PP for a three day course of therapy and of primaquine for a 14 day course of therapy. The soldier may take these medications on his own cognizance. The additional visits and sampling described in Table 8 are not required but will be followed if resources are available.

5.8 Final Study Visit

The final study visit will occur approximately 24 weeks after return for normal duty at their home base, if resources allow. However, the final study visit is not required for safety reasons because soldiers will already have been surveilled for 24 weeks after their redeployment to their home base, enough time to identify relapsing *P. vivax* infections. If the final study visit does take place, evaluations to be done during the final study visit are listed in the Study Procedures/Evaluations section (see **Table 7**). If possible, these will include the full parasitology

assessment (including 5 mL for in-depth genome sequencing) but all blood collection and testing is optional, depending on staffing and resources available.

5.9 Early Termination Visit

If a participant wishes to end their participation early and is willing to have evaluations performed, a physical examination will be done and 10 mL venous blood may be drawn for clinical blood analysis, thick and thin smear, samples for qPCR, samples for genome sequencing, and/or for immunology assays.

Investigators will make every effort to continue follow-up visits for any participant who has received one or more vaccinations for the duration of the study even if it is determined that subsequent vaccination should not be administered. Participants who do not receive subsequent vaccinations will not be replaced by new participants.

5.10 Unscheduled Visit

Unscheduled visits will prompt a history and targeted physical examination (as needed) to ascertain cause of illness. Clinical laboratory tests such as a blood smear and any other medically indicated diagnostic or therapeutic procedures will be done as appropriate. These visits will be recorded as observations in the CRFs.

5.11 Active Surveillance

Active surveillance to detect AEs and acute malaria will consist of the scheduled visits during the vaccination phase of the trial, with the visits scheduled for every two weeks during the period of exposure to malaria in eastern Indonesia. Post-exposure active surveillance on the home base consists of a blood smear examination and qPCR sample every 4 weeks.

Participants who present with fever will be evaluated for malaria and other potential etiologies based on their symptoms and clinical judgment of the investigators and clinical staff. Guidelines for evaluation and management of fever in adults have been established by the Indonesian Ministry of Health and will be followed.

Blood smears from asymptomatic individuals will be read retrospectively later in the deployment or after deployment is finished. If they are found to be positive, the participant will be contacted and evaluated for malaria infection as appropriate (some will have already presented with clinical malaria and already treated).

5.12 Passive Surveillance

Passive surveillance in this study amounts to accessibility of study participants to clinical care when ill. Study participants designated as “accessible” will have direct access to that care 24/7 while in the field. Study participants designated as “remote” have the same access but to a paramedic operating under the supervision of the Site Medical Officer at the primary field study center.

6 STUDY PROCEDURES/EVALUATIONS

6.1 During and After Deployment

6.1.1 Malaria event

- Acute malaria of Pf or Pv confirmed by two expert microscopists.
- Record vital signs and symptoms in the CRF.
- Collect blood samples as specified in **Table 7** and **Table 8**.
- Store blood at 4 degrees centigrade (or on ice) and transport to laboratory within 24 hours.
- Administer first dose of DHA-PP (or other appropriate drug) and observe for 30 minutes for vomiting.
- At approximately the same time on the next two days, directly administer remaining doses of DHA-PP (or other appropriate drug), recording all in the CRF.
- Note that treatment for latent malaria is not administered until return to home base .
- Confirm recovery from malaria by clinical examination on days +1, +2, +7, +14, +21 and +28 days after diagnosis. Blood smears will be taken on days +7 and +28 (blood smears from remote site read retrospectively).
- If treated study participant has a recurrent infection at any visit through +28 days, consult study physician for recommendations regarding treatment.
- Study participant resumes routine follow-up visits approximately +28 days post-diagnosis to allow continued surveillance for Pf and Pv malaria parasitemias and clinical infections.

6.1.2 Surveillance and follow-up during transit to home base

- The research team with CRFs will accompany the battalion onboard ship for the approximate two-week journey.
- Participants will remain under the schedule of routine follow-up until a final visit to complete the exposure period within +10 days of departure, at which time they will be considered no longer at risk of a primary attack of malaria and the field phase of this trial concludes.
- Any attacks occurring onboard the ship shall be managed as described above.

6.1.3 Surveillance and follow-up after return to home base

- The research team with CRFs will accompany the battalion back to their home base.
- Passive case detection will be used through out the post-redeployment follow-up period.
- If resources allow, there will additionally be 4-weekly visits and examinations (as opposed to 2-weekly in Papua). A post-exposure home leave is routine and anticipated so the schedule for each participant will need to be flexible. The first surveillance visit will be conducted as soon as possible after arrival, with a target of completing all participant first visits within 28 days. See **Table 7** and **Table 8** for the blood tests to be drawn and follow-up schedule. The first and last post-deployment visits will include the 5 ml venous sample for genome sequencing in addition to the routine parasitology samples, if resources allow.

- Confirmed attacks of Pv malaria will be treated with DHA-PP administered concurrently with primaquine (0.5 mg/kg/day, 14 days). If resources allow, this will be done under direct observation, but this is not required.
- Venous blood is collected as described in **Table 7** and **Table 8** prior to treating Pv malaria attacks. Again, the collection of research samples is resource-dependent.
- Participants completing twenty-four weeks of follow up have reached the end of their participation as study participants in this trial.
- Venous blood will be collected as described in **Table 7** at the last study visit (resource dependent). The CBC and chemistry tests at this visit are primarily motivated by scientific, not safety reasons. Specifically, after six months back at home base, there are no residual safety concerns related to either immunization, which took place more than a year before, or to deployment in Papua, which ended six months before.

6.2 Clinical Evaluations

6.2.1 Medical history

A medical history from childhood onward will be taken with special attention to recurrent infections that suggest immune suppression, previous history of splenectomy and prior vaccine reactions. Systems to be reviewed include head/eyes/ears/nose/throat, pulmonary, cardiovascular, gastrointestinal, genitourinary, skin, musculoskeletal, neurological, allergy/immunology, endocrine, and hematological. During the study, any new findings that are identified outside of the solicited/unsolicited adverse event collection periods will be recorded in the clinical event CRF (periods defined in **Figure 4**).

6.2.2 Medications history

As part of the medical history, a medications history for the past 28 days will be taken with special attention to immunosuppressive medications including corticosteroids. Participants will be queried about medications prescribed by a clinician, over-the-counter medications, and any homeopathic or traditional medications. These medications will be reviewed before enrollment and throughout the study with special attention for prohibited medications.

6.2.3 Electrocardiogram (ECG)

As part of the screening process, an ECG will be performed for potential participants and read by study physicians. Participants with prolonged QT/QTc interval and those with other findings indicative of clinically significant cardiac disease will be excluded.

6.2.4 Physical examination

As part of the screening process, study clinicians will perform a physical examination. Vital signs, including axillary temperature, blood pressure, respiratory rate and pulse, will be assessed with the physical examination. Subsequent physical examinations may be more targeted based on the need to evaluate for vaccine reactogenicity or any complaints noted by a participant, including suspected AE evaluation. Vital signs to be assessed at all follow-up visits are outlined by visit in the daily study procedures section.

6.2.5 Adverse events (Aes) assessments

PfSPZ Vaccine: Surveillance for solicited local AEs will be done retrospectively (on the day of vaccination, all participants will be informed to look at the injection site for +2 days after each dose and at the next visit after vaccination, the study team will ask the participant if there are any complaints in the area around the injection site); for solicited systemic AEs will be done for +7 days after each dose of PfSPZ Vaccine; and for unsolicited AEs for +14 days after each dose of PfSPZ Vaccine. Collection of solicited and unsolicited AEs will be done before participants leave on the day of vaccination and at scheduled visits post vaccination. SAEs will be recorded throughout the study.

PfSPZ-CVac: *PfSPZ Challenge and CQ*: Surveillance for solicited local AEs will be done retrospectively (on the day of vaccination, all participants will be informed to look at the injection site for +2 days after each dose and at the next visit after vaccination, the study team will ask the participant if there are any complaints in the area around the injection site); for solicited systemic AEs will be done continuously from Day -2 to Day 69 (+7 days after the last dose of CQ); and for unsolicited AEs from Day -2 to Day 71 (+14 days after the last dose of PfSPZ Challenge). Collection of solicited and unsolicited AEs will be done before participants leave on the day of vaccination and at scheduled visits post vaccination. SAEs will be recorded throughout the study.

6.2.6 Health care provision

Upon recruitment, study participants will have access to routine and acute care by the research team and its Indonesian Army medical component partners 24 hours a day, 7 days a week until the end of study participation. This is fully described in section 1.3 above (risks & benefits of the trial).

In the event of unexpected severe illness or trauma every resource available shall be mobilized to get the patient to the level of care required as quickly as possible. We acknowledge and anticipate that rapid access to adequate care may not occur in some instances, especially at the remote sites involved in this study. In anticipation of a medical emergency at those sites, the research team will deliberately place paramedics at each in order to be able to stabilize the patient via supervision from the Site Medical Officer.

6.3 Laboratory Evaluations

The investigators will maintain detailed SOPs for all laboratory assays at the study site and central laboratories at study center headquarters in Jakarta. These SOPs will include sample collection, handling (e.g. serum separation), labeling, preservation (e.g. preparation and storage of PBMC and material for genomic analyses), storage, transport, and shipping. All staff and investigators will be trained in the SOPs relevant to their duties and sign copies of the SOPs to document this training. Copies of SOPs will be available for inspection and review by study monitors. The general methods that will be used are summarized in the following section.

6.3.1 Clinical laboratory evaluations

Routine safety laboratory testing including clinical blood analysis will be performed at the temporary study clinical laboratory at the home base site, with back up testing available at the Klinik Utama Prodia Pekanbaru. Results will be entered into the study central database according to a data entry SOP. Screening laboratory values will guide study clinicians considering initial and ongoing eligibility for vaccinations. If baseline clinical lab values fall within Grade 1 parameters, then a laboratory abnormality is documented only if there is a change (increase) to a severity of Grade 2 or higher parameter for the clinical lab. The designation of clinical significance by the PI or designee will also be taken into consideration. Laboratory abnormalities will be followed up as clinically indicated, in most cases by repeating the test. The follow-up plan will be tailored to each individual case.

6.3.1.1 Hematology and biochemistry

Tests for hematological and blood biochemistry will be performed for safety assessment in all participants. Hematology tests will include a complete blood count (CBC) and biochemistry will include creatinine and ALT for renal and liver functions. At screening only, additional liver function tests (bilirubin, and gamma GTP) will be done, as will a glucose measurement. Hereafter these tests are referred to generically as “clinical blood analysis”.

PfSPZ Vaccine: Clinical laboratory analysis will be done at screening, on the day of first immunization (with -2 days allowed window) and +14 days after the third immunization. Clinical laboratory analysis will also be performed at the end of the exposure and (optionally) post exposure periods.

PfSPZ-CVac: Clinical laboratory analysis will be done at screening, on the day of first immunization (with -2 days allowed window) and +14 days after the last immunization. Clinical laboratory analysis will also be performed at the end of the exposure and (optionally) post exposure periods.

The Principal Investigator will maintain laboratory reference intervals in the study file, and copies will be made available upon request to the Research Monitor, clinical monitors and Sponsor. Study-wide hematology and serum biochemistry assays will be performed at the clinical laboratory at the study site. During deployment, hospital-based or commercial laboratories will be used if needed for laboratory testing of individual soldiers with clinical concerns.

6.3.1.2 Urinalysis

A urinalysis will be performed at screening.

6.3.1.3 Troponin

A serum sample for retrospective troponin analysis will be collected at baseline prior to the first vaccination from all participants and stored to run in a comparative assay if needed.

6.3.1.4 Glucose-6-phosphate dehydrogenase (G6PD)

G6PD deficiency will be assessed at screening, but not applied as a basis for exclusion.

6.3.1.5 Malaria Smears

Both thick and thin blood smears will be obtained during all three phases of the study – Pre-exposure; Exposure; Post-exposure – for the purposes of screening, and of detecting vaccine-derived acute malaria, primary attacks of acute malaria, and relapses due to Pv malaria, respectively. A participant will be declared as being malaria positive and immediately treated only after a second expert microscopist confirms the positive diagnosis (striving to complete this within 1 hour of the first diagnosis), or after a positive RDT at remote sites. Standard operating procedures are followed to assure uniform and high-quality malaria thick and thin smear preparation. Accurate identification of species and quantification and staging will be assured based on SOPs. Malaria microscopists will have been trained and certified as expert in malaria diagnostic methods prior to active reading of slides in this trial. Blood for malaria thick and thin blood smears will be obtained at the following times:

PfSPZ Vaccine: At time of screening, at time of first immunization, 2 weeks after the last immunization, on arrival to the field site and every 4 weeks during Exposure, every 4 weeks during Post-exposure, and at any time during Exposure or Post-exposure that a participant has symptoms that could be malaria.

PfSPZ-CVac: At time of screening, at time of each immunization, +7, +8, +9, and +12 days after the first and second immunizations, +7, +8, +9, and +14 days after the third immunization, on arrival to the field site and every 4 weeks during Exposure, every 4 weeks during Post-exposure including the last study visit, and at any time during Exposure or Post-exposure that a participant has symptoms that could be malaria.

Timing for reading blood smears: Blood smears will be read at the time of screening and must be negative for the research participant to be eligible for inclusion. If positive, the research participant will be treated prior to immunization, with a minimum five day gap between last dose of DHA-PP and first vaccination. At any point during the trial, blood smears obtained from febrile research participants will be read as soon as possible (unless they are at remote postings), ideally within 4 hours. Blood smears from research participants not suspected of being infected with malaria will be read retrospectively later during deployment or after redeployment. In Groups 3 and 4, blood smears will be read generally within 48 hours on +7, +8, +9 and +12 days after the first and second PfSPZ Challenge injections and +7, +8, +9, and +14 days after the third PfSPZ Challenge injection. For participants in remote locations blood smears from symptomatic individuals are read after delivery to the microscopy team, which may be days to weeks after collection and slide preparation. Blood smears done at the last study visit, if positive, will result in the study team contacting the research participant for treatment.

Thin blood smears: The thin blood smear will not be read if the thick blood smear is negative. If the thick blood smear is positive, the thin blood smear will be used for two purposes:

1. Species identification: Thin blood smears will be used to aid in immediate malaria species identification based on parasite and red blood cell morphology, when the density is high enough for parasites to be seen on thin blood smear. This information will help with determining the proper treatment regimen. However, definitive species identification for endpoint determination will be made retrospectively by molecular assays (see next section on qPCR).

2. Quantification of high density parasitemia: When parasite densities on the thick blood smear are above 25 asexual parasites in the first high-powered field of a 0.2 mm objective lens (>15,625 parasites/ μ L blood or >0.3% parasitemia), the thin blood smear will be used to calculate

percent parasitemia. As above, this will provide guidance on the proper treatment regimen, since hyperparasitemia is a criterion for severe malaria, and will also aid in following the response to treatment in participants with parasite densities $>15,625$ parasites/ μL blood or $>0.3\%$ parasitemia given that documenting a substantive drop in parasite density by 48 hours after the first antimalarial drug administration is an important aspect of clinical follow-up.

6.3.1.6 qPCR

A standard nested-qPCR for detection of Plasmodium species nucleotides will be done at the EIMB in Jakarta for multiple reasons:

1. Exclusion of parasitemia before, during and after immunizations during the Pre-exposure period.
2. Exclusion of parasitemia at the beginning of the Exposure period (optional).
3. Confirmation of parasitemia detected by microscopy during Exposure and Post-exposure*. Whenever a blood smear is made, a sample or samples for qPCR will be taken. If resources allow, samples associated with a positive blood smear will be assessed by qPCR. The qPCR will be used to confirm the microscopic diagnoses and perform an exploratory analysis. Reconciliation of discordant microscopy-qPCR results will be attempted by repeating independent and blinded expert microscopic reads, the qPCR testing, or both (a negative blood smear in the setting of low parasitemia detected by qPCR will not be considered discordant).
4. Determination of parasitemia after each dose of PfSPZ Challenge (+7, +8, +9 and +12 days after the first and second doses of PfSPZ Challenge, and +7, +8, +9 and +14 days after the last dose of PfSPZ Challenge) (optional).
5. To ascertain if any participants may need to be contacted and treated after the end of the study due to ongoing parasitemia that was not detected by blood smear (optional).

* Using PCR to confirm study endpoints: In addition to (or in place of) the standard qPCR described above, a different qPCR will be run (if resources are available) to confirm all results serving as primary and secondary trial endpoints (cases of clinical malaria or parasitemia identified by blood smear during the deployment or post-deployment study periods). This second qPCR, developed at the laboratory of Dr. Sean Murphy, University of Washington Medical Center, is the only FDA-qualified biomarker of malaria infection. Additional details are provided on the FDA's website <https://www.fda.gov/drugs/biomarker-qualification-program/fda-reviews-qualified-biomarker-plasmodium-18s-rnrdna>. The assessment will include confirmation of the malaria species present in the sample, since it is anticipated that mixed infections will be common and these can be difficult to diagnose from blood smears.

The following samples will be taken for qPCR:

PfSPZ Vaccine: At time of screening, at time of first immunization, 2 weeks after the last immunization, on arrival to the field site and every 4 weeks during Exposure, every 4 weeks during Post-exposure, and at any time during Exposure or Post-exposure that a participant has symptoms that could be malaria (may only run if blood smear is positive).

PfSPZ-CVac: At time of screening, at time of each immunization, +7, +8, +9, and +12 days after the first and second immunizations, +7, +8, +9, and +14 days after the third immunization, on arrival to the field site and every 4 weeks during Exposure, every 4 weeks during Post-exposure including the last study visit, and at any time during Exposure or Post-exposure that a participant has symptoms that could be malaria (may only run if blood smear is positive).

Timing for performing qPCR: All screening samples will be run promptly, due to the requirement to treat any soldiers who are qPCR positive prior to the first immunization. All other samples will be run retrospectively.

6.3.1.7 RDT

Rapid Diagnostic Tests (RDTs) will be used to diagnose malaria in remote sites. The RDT that will most likely be used in this trial is SD BIOLINE Malaria Ag P.f/P.f/P.v. (Cat. No.: 05FK120). This test is based on detection of Pf-HRP2, Pf-pLDH and Pv-pLDH. It will therefore also capture isolates with Pf-HRP2 gene deletion. Sensitivity and specificity provided by the Manufacturer are 99.7% and 99.3% for Pf-HRP2, 97.4% and 99.3% for Pf-pLDH, and 95.5% and 99.3% for Pv-pLDH, respectively. False-negative result rates are expected to be 0.3% for Pf (or less, given that the assay measures 2 enzymes) and 0.5% for Pv. An alternative RDT may be used if the SD BIOLINE is not available.

6.3.2 Special assays or procedures

6.3.2.1 Serology assays

Assays for antibodies against Pf circumsporozoite protein (PfCSP) will be performed at the EIMB following SOPs for methods that have been used for the previous trials of our vaccines. The methods have been well described [13]. In addition, assays for antibodies against killed PfSPZ, and live PfSPZ, and potentially for other Pf proteins and parasite stages, and for PvCSP may be done. The assays include ELISA, automated immunofluorescence assay (aIFA) and automated inhibition of sporozoite invasion assay (aISI). These studies will be done on samples collected at baseline (pre-immunization), +14 days after the third immunization, at the beginning of the Exposure period and at the beginning and end of the Post-Exposure period.

The EIMB and its collaborators at OUCRU ID, Sanaria, and the Walter & Eliza Hall Institute at Melbourne, Australia (under sponsorship of the Bill & Melinda Gates Foundation) may analyze a specific subset of sera collected under this protocol: post-exposure baseline sera will be segregated into two classes; 1) participants assigned to NS placebo who went on to relapse with Pv during post-exposure surveillance; and 2) participants assigned to NS vaccine who did not relapse with Pv during that period. In other words, they will conduct a search for serological markers or correlates specific for hypnozoite carriage employing a large number of recombinant Pv peptides in microarray plates.

6.3.2.2 Cell-mediated immunity

Peripheral blood mononuclear cells (PBMC) will be cryopreserved and transported to the PRBME-BRIN/OUCRU ID in Jakarta for CMI assays. At a minimum, samples will be collected at baseline, and +12 and +14 days after the first and third immunizations, respectively for the PfSPZ-CVac group and at baseline, and +14 days after the second and third immunizations for the PfSPZ

Vaccine group. Specimens may also be collected at the end of the Exposure period, depending on resources. Decisions regarding what assays will be conducted will be made based in part on outcomes from the trial. Most of the cellular analyses will be done based on the results of ongoing studies at other sites. However, it is anticipated that flow cytometry and B cell assays will be done as in previous trials [9, 10, 12, 13].

6.3.2.3 Genome sequencing and genotyping

In preliminary studies aimed at identifying antigen(s) responsible for protective immunity elicited by PfSPZ Vaccine and PfSPZ-CVac, we will conduct parasite genome-wide association studies to identify Pf genetic loci selected by vaccination with PfSPZ Vaccine and/or PfSPZ-CVac and associated with vaccine escape at EIMB in Jakarta. Parasite DNA extracted from leukocyte-depleted blood collected during clinical episodes and follow-up visits will be participated to genome sequencing and analyses performed to measure vaccine selection and allele-specific efficacy.

Whenever venous blood is obtained for research purposes from participants having acute malaria, blood will be processed and stabilized for subsequent DNA extraction and genotyping. When sufficient volumes are available after blood is allocated for other uses, pellets blood may be cryopreserved for later expansion in culture for further parasite characterization by molecular and in vitro methods such as growth inhibition assays. Whenever malaria blood smears are obtained, a few drops (less than 0.5 mL) of blood will be blotted onto filter paper and preserved for parasite genome sequencing, and at key points in the study (see **Table 7**) a larger, 5 mL sample will also be collected.

6.3.3 Specimen reparation, handling and shipping

Detailed SOPs are maintained for these activities. Briefly, routine blood samples are obtained at the research clinic and processed in the sample-processing laboratory according to SOPs. Filter paper blood samples are stored at room temperature in sealed desiccant pouches. Sera, plasma and cells are frozen in freezers or in liquid nitrogen containers according to SOPs. Frozen samples will be transported to the central immunology laboratory in Jakarta, and either stored there in temperature-monitored freezers, a liquid nitrogen storage system. Blood samples taken from accessible participants with malaria will be stored in refrigeration or ice for less than 24 hours prior to transport to the hospital-based laboratory for processing, labeling, temporary storage in freezers or liquid nitrogen, and finally, transport to the laboratory in Jakarta.

The Investigators will maintain detailed SOPs for vaccine transport, storage, preparation, reconstitution and administration. All staff and investigators will be trained in the SOPs relevant to their duties and will sign copies of the SOPs to document this training. Copies of SOPs will be available for inspection and review by the CDMRP, Sanaria and study monitors. During the study SOPs may be modified to improve them and new SOPs may be developed as needed to improve operations and ensure adherence with the protocol.

7 ASSESSMENT OF SAFETY

7.1 Specification of Safety Parameters

The primary safety outcome measures for this trial are (see section 2.3.2, primary outcome measures):

- The number of SAEs related to vaccination or placebo administration during active participation in the trial.
- The number and severity of solicited AEs occurring within 7 days (PfSPZ Vaccine) or 14 days (PfSPZ-CVac) of each administration of investigational product (IP) related to vaccination or placebo administration.
- The number and severity of unsolicited AEs occurring within 14 days (PfSPZ Vaccine, PfSPZ-CVac) of each administration of investigational product (IP) related to vaccination or placebo administration.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

An adverse event (AE) includes any noxious, pathological or unintended change in anatomical, physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory-detected changes occurring during the periods of AE collection in the clinical study whether or not associated with the study product and whether or not considered related to the intervention. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, or drug interaction. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation will not be considered adverse events. Discrete exacerbations of chronic conditions that are deemed to be different than regularly sustained day-to-day fluctuations will be reported as adverse events in order to assess changes in frequency or severity.

AEs will be documented in terms of a medical diagnosis. When this is not possible, the AE will be documented in terms of signs and/or symptoms observed by the investigator or reported by the participant at each study visit. All AEs occurring during specified periods while on study will be documented appropriately regardless of relationship. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a participant before the start of the study will be recorded as an AE if deterioration or exacerbation in the condition occurs during the study. Significant AEs occurring outside the designated periods of collection will be recorded on the clinical event CRF.

Any hospitalization other than the planned inpatient evaluation for the malaria event will be considered a serious adverse event (SAE). Information to be collected include event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event. All AEs will be graded for severity and relationship to study product and followed to adequate resolution or stabilization. Laboratory abnormalities will be tallied separately from signs, symptoms or medical diagnoses.

7.2.1 Solicited adverse events

Both local and systemic solicited AEs will be collected at specified time points during the trial. The time points are delineated in **Figure 4** below. Documentation of these AEs will occur during regularly scheduled clinic visits, where the research participant will be questioned about current health status and retrospectively about health issues since the prior clinic visit.

Local solicited AEs (at the injection site). At each post-vaccination follow-up visit where it is noted in **Table 7**, a trained member of the research team will question the participant regarding a prescribed list of local AEs (**Table 9**), and examine the prior injection site(s) for any obvious sign of injury or infection. The findings will be noted in the CRF and positive findings may be documented with a photograph to be entered into the CRF.

Systemic solicited AEs: At each post-vaccination follow-up visit where it is noted in **Table 7**, a trained member of the research team will question the participant regarding a prescribed list of systemic AEs (**Table 9**).

Solicited AE lists are provided in **Table 9**. The list of local AE's (Block 1) is the same for both PfSPZ Vaccine and PfSPZ-CVac. For systemic AE's, there is a core list (Block 2) that applies to both PfSPZ Vaccine and PfSPZ-CVac. For those participants receiving PfSPZ-CVac an *additional* list of systemic AEs (Block 3) will be solicited beginning two days before PfSPZ-CVac administration (Day -2) to monitor for the effects of CQ and later for the effects of any parasitemia experienced on days +7, +8 and +9 after each injection of PfSPZ Challenge. Solicitation of these AEs will be continuous and will be collected at each visit from Day -2 through Day 69 (+7 days after the last dose of CQ for those research participants receiving PfSPZ-CVac/NS).

Solicitation for systemic AEs in research participants receiving PfSPZ Vaccine will be done for the +7 day period following each injection with PfSPZ Vaccine and will be collected at each +7 day post vaccination visit. Solicitation for systemic AEs in research participants receiving PfSPZ Vaccine/NS is therefore more limited than before PfSPZ-CVac/NS research participants. This is because (a) there is no need to follow them for transient parasitemia; (b) they are not receiving CQ every week.

The period of time where each participant is monitored for solicited AE's is provided in Figure 4.

PFSPZ-CVAc / Normal Saline Groups

CDSC day numbers

Day 1 Day 29 Day 57

PERIOD OF TIME COVERED FOR SOLICITED LOCAL AEs

PERIOD OF TIME COVERED FOR SOLICITED SYSTEMIC AEs

PERIOD OF TIME COVERED FOR UNSOLICITED AEs

PERIOD OF TIME COVERED FOR UNSOLICITED AEs

Clinic Visits

Days relative to vaccination

AEs assessed retrospectively to time of prior clinic visit; also assessed to time of PFSPZ Challenge injection earlier the same day, if it is Day 1, 29 or 57.

AEs assessed retrospectively to time of CQ administration earlier the same day

PFSPZ Vaccine / Normal Saline Groups

CDSC day numbers

Day 1 Day 8 Day 29

PERIOD OF TIME COVERED FOR SOLICITED LOCAL AEs

PERIOD OF TIME COVERED FOR SOLICITED SYSTEMIC AEs

PERIOD OF TIME COVERED FOR UNSOLICITED AEs

PERIOD OF TIME COVERED FOR UNSOLICITED AEs

Clinic Visits

Days relative to vaccination

AEs assessed retrospectively to time of prior clinic visit; also assessed to time of PFSPZ Vaccine injection earlier the same day, if it is Day 8.

AEs assessed retrospectively to time of PFSPZ Vaccine injection earlier the same day

During surveillance, following malaria symptoms will be solicited every two weeks or whenever malaria is suspected: fever, subjective fever, headache, fatigue, malaise, chills, arthralgia (joint pain), myalgia (muscle pain), dizziness, rigors, sweats, cough, nausea, vomiting, abdominal pain, diarrhea, chest pains, palpitations, shortness of breath.

Table 9. List of Solicited AEs to be monitored

Categories of AEs		Symptoms and Signs	PfSPZ-CVac	PfSPZ Vaccine
Block 1	Local Solicited AEs At injection site	<u>By palpation:</u> 1 • Tenderness* to palpation 2 • Induration to palpation <u>By visualization:</u> 3 • Bruising / extravasated blood 4 • Erythema 5 • Swelling (by lateral visualization) <u>By history:</u> 6 • Pain 7 • Pruritus	By medical history and exam at time of dosing, and then for +2 days.	By medical history and exam at time of dosing, and then for +2 days.
Block 2	Systemic Solicited AEs CORE LIST	Fever $\geq 37.5^{\circ}\text{C}$ Allergic reaction (<i>rash, urticaria, pruritus, edema</i>) Headache Subjective fever Fatigue Malaise Chills Myalgia Arthralgia	By medical history and exam at each clinic visit during vaccination, starting on the day of first CQ dose* through +7 days after the last CQ dose (Day -2 through Day 69).	By medical history and exam on the day of each vaccine dose*, and 7 days after each dose. <i>For PfSPZ Vaccine, only Block 2 AEs will be asked,</i>
Block 3	Systemic Solicited AEs Post CQ Administration <i>In addition to Core List</i>	Dizziness Rigors Sweats Cough Nausea Vomiting Abdominal pain Diarrhea Chest pain Palpitations Shortness of breath Tinnitus Blurred Vision Photosensitivity Insomnia Pruritus Anxiety Confusion	<i>For PfSPZ-CVac, both Block 2 and Block 3 AEs will be asked at each clinic visit.</i>	Not applicable

* Solicited AEs will be reviewed after CQ is dosed, or after the vaccine is administered.

7.2.2 Unsolicited adverse events

Unsolicited AEs will be identified through open-ended questioning such as “do you have any other illness or medical complaint?” This will be asked to prompt the identification of additional AEs (unsolicited AEs) after reviewing the solicited list, or as the sole means of collecting AE data during visits when solicited AEs are not being collected. Unsolicited AEs will be collected for +14 days after each PfSPZ Vaccine vaccination, and continuously for PfSPZ-CVac from Day -2 to Day +70 (see **Figure 4**). However, to address the primary safety outcome variable, they will be tallied for +14 days after each vaccination with PfSPZ-CVac, as the AEs recorded at other times are more likely to relate to chloroquine or other causes than to the injected PfSPZ. A syndromic classification will be used for documenting unsolicited AEs – e.g., cough, nasal congestion, sore throat should be combined into upper respiratory tract infection. Thus, the term for the unifying diagnosis is recorded as the AE, not each individual sign or symptom whenever applicable.

AEs spontaneously reported by study participants will also be fully documented and any changes in health history outside of the reporting period will be recorded in the clinical event CRF. The signs and symptoms associated with malaria itself when diagnosed during surveillance will be recorded on specific case report forms.

7.2.3 Assessment of severity of adverse events

All AEs will be assessed by the Principal Investigator or designee based on documented report of the study participant and clinical observation. The assessment scale used for all AEs in this protocol are shown in **Tables 10** and **11**. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent require documentation of initial onset and final duration of the event.

Table 10. Assessment of solicited local AE severity

Adverse Event	Grade	Severity Assessment
Pain at injection site		
Pruritus at injection site	1	Does not interfere with activity
	2	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity
	3	Any use of narcotic pain reliever or prevents daily activity
Tenderness at injection site	1	Discomfort only to touch – does not interfere with activity
	2	Discomfort with movement – interferes with activity
	3	Significant discomfort at rest – prevents daily activity
Erythema/redness at injection site (greatest single diameter)	1	2.5 to 5 cm and does not interfere with activity
	2	5.1 to 10 cm or interferes with activity
	3	>10 cm, necrosis or exfoliative dermatitis or prevents daily activity
Swelling		
Bruising	1	2.5 cm to 5 cm and does not interfere with activity

Induration at injection site (greatest single diameter)	2	5.1 cm to 10 cm or interferes with activity
	3	>10 cm or prevents daily activity

Table 11. Assessment of severity of solicited systemic AEs and unsolicited AEs

Adverse Event	Grade	Severity Assessment
Fever (axillary temperature*)	1	37.5-38.4°C
	2	38.5-38.9°C
	3	≥39.0°C
All other systemic AEs listed in Blocks 2 and 3 of Table 9 and any unsolicited AE	1	Mild; no interference with activity
	2	Moderate; some interference with activity
	3	Severe; significant; prevents daily activity

*Axillary temperature will be recorded at the time of the clinic visit. If additional temperature measurements are recorded at another time of the day, the highest temperature will be recorded.

7.2.4 Assessment of causality of adverse events

The clinician's assessment of an AE's relationship to test vaccine or study drug will be part of the documentation process. The clinician will assess the relationship between study product or procedures and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of underlying diseases, concomitant therapy, other risk factors, other diseases, other events and the temporal relationship of the event to inoculation with PfSPZ Vaccine, PfSPZ Challenge or administration of malaria chemoprophylaxis will be considered and investigated. The relationship/ causality of the AE to the study procedures will be assessed and categorized by the investigator using the following guidelines (**Table 12**):

Table 12. Causality gradings

Not related	A relationship to the administration of the IP cannot be reasonably established; another etiology is known to have caused the adverse event or is highly likely to have caused it.
Unlikely	A relationship to the administration of IP is unlikely; however, it cannot be ruled out.
Possibly	There is a potential association between the event and administration of the IP; however, there is an alternative etiology that is more likely.
Probably	Administration of the IP is the most likely cause; however, there are alternative reasonable explanations, even though less likely.
Definitely	An association exists between the receipt of IP and the event. An association to other factors has been ruled out.

The investigator may change his/her opinion of causality in light of follow-up information and enter a revised AE report into the CRF. The Investigator and Sponsor may both provide causality assessments. The Sponsor is ultimately responsible for classifying the relationship, taking into consideration the investigator's assessment. When a regulatory or other authority requests distinct classification of AEs into either related or unrelated, without intermediate categories, "not related" and "unlikely related" will be combined as "unrelated" and "possibly related," "probably related," and "definitely related" will be combined as "related."

7.2.5 Evaluation and assessment of vital signs abnormalities

Pulse, blood pressure, and respiration are assessed at each visit and graded according to the following criteria (Table 13).

Table 13. Vital signs toxicity grading

Vital Signs*	Grade 1	Grade 2	Grade 3
Tachycardia- beats per minute	101-115	116-130	> 130
Bradycardia- beats per minute	45-49	40-44	< 40
Hypertension (systolic) mm Hg	141-150	151-160	> 160
Hypotension (systolic) mm Hg	85-89	80-84	< 80
Tachypnea- breaths per minute	21-25	26-30	> 30

7.2.6 Evaluation and assessment of clinical laboratory findings

Abnormalities in clinical laboratory testing will be followed up according to the national guidelines of Indonesia (Tables 14 and 15). The clinician will assess any abnormal laboratory finding as clinically significant or non-clinically significant per their medical judgment. In general, laboratory abnormalities that require intervention will be classified as clinically significant. Laboratory abnormalities will be tallied separately from other AEs.

Table 14. Normal laboratory blood and urine values for Indonesia.

Blood Hematology Test	
Parameter	Normal Values
Hematocrit (Hct) – male	40% - 52 %
Hemoglobin (Hb) – male	13.2 – 17.3 g/dL
Platelets	150 x10 ³ - 440 x10 ³ /μL
WBC	3.8 x 10 ³ - 10.6 x 10 ³ /μL

percentage

Granulocytes	53 – 80%
Lymphocytes	25% - 40%
Monocytes	2% - 8%

RBC

male	4.4 – 5.9 x 10 ⁶ /μL
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RBC Measurements

Mean corpuscular volume (MCV)	80 – 100 (fL)
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Mean corpuscular hemoglobin (MCH)	26– 34 pg
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Mean corpuscular hemoglobin concentration (MCHC)	32 – 36 g/dL
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Reticulocytes	0.5-1.5%
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Blood Chemistry Test

Parameter	Normal Values
Alanine aminotransferase (ALT)/GPT	0-50 U/L
Gamma glutamil transferase (GGT)	0-30 U/L
Bilirubin serum (total)	0.1 – 1.0 mg/dL
Bilirubin, conjugated (direct)	0 – 0.2 mg/dL
Creatinine	0.62– 1.10 mg/dL (male)
Glucose	70 – 120 mg/dL

Urinalysis/ Dipstick test

Parameters	Normal Values
Glucose	Negative
Bilirubin	Negative
Ketones	Negative
Specific Gravity	1.015-1.025 g/ml
Blood	Negative
pH	4.5-8
Protein	Negative
Urobilinogen	Normal (0.2-1.0 E. U. /dL)

Nitrites Negative
Leukocytes Negative

Source:

- Conversion Table of SI-Conventional Units and Reference Values for Adults-Children Clinical Laboratory Parameters, Indonesian Association of Clinical Pathology Specialists, Jakarta Branch, First Edition. 2004 (*Tabel Konversi Satuan SI-Konvensional dan Nilai Rujukan Dewasa-Anak Parameter Laboratorium Klinik, Perhimpunan Dokter Spesialis Patologi Klinik Indonesia Cabang Jakarta, Cetakan Pertama. 2004*)
- Clinical Data Interpretation Guidelines, Ministry of Health Republic of Indonesia. 2011 (*Pedoman Interpretasi Data Klinik, Kementerian Kesehatan Republik Indonesia. 2011*)

Table 15. Laboratory toxicity grading

HEMATOLOGY*				
	Grade 1	Grade 2	Grade 3	
Hemoglobin Male	12.5 – 13.1 gm/dL	10.5 – 12.4 gm/dL	≤10.5 gm/dL	
Platelets	125,000 - 149,999/mm ³	100,000 - 124,999/mm ³	≤100,000/mm ³	
WBCs (increase)	10,601- 15,000/mm ³	15,001-20,000/mm ³	>20,000/mm ³	
WBCs (decrease)	2500 – 3799/mm ³		1500 – 2499/mm ³	≤1500/mm ³
CHEMISTRIES*				
	Grade 1	Grade 2	Grade 3	
Creatinine	1.11-1.70 mg/dl (male)	1.71-2.00 mg/dl	>2.00 mg/dl	
ALT	>1.0x and ≤ 2.5x ULN	> 2.5x and ≤ 5x ULN	> 5x ULN	

*Grading scales derived from "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", USFDA, 2007, and adjusted to locally derived normal ranges.

7.2.7 Serious adverse events

An AE or suspected adverse reaction will be considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening illness,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- Important medical events that may not result in death, be life threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment

they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An AE is considered “life threatening” if, in the view of either the investigator or Sponsor, its occurrence places the patient or participant at immediate risk of death. It does not include an AE, had it occurred in a more severe form, might have caused death.

All SAEs will be assessed for severity and relationship to study product and alternate etiology provided (if not related to study product) by the principal investigator or sub-investigator; recorded on the appropriate SAE form and/or CRF. The causality of SAE’s for which the PI assesses possibly, probably or definitely related to the study product will be reviewed by Sanaria, the RM, and the SMC prior to notification of the applicable ethical and institutional review committees using the algorithm described below on the study pause section. The Sponsor will have final decision as to the causality of all SAEs. All SAEs will be followed through resolution by the principal investigator or sub-investigator.

7.2.8 Reporting procedures

7.2.8.1 Adverse events

All AEs will be documented from the first study intervention through 14 days after the last dose of vaccine. SAEs will be documented through the end of the study follow-up period. All AEs will be captured on the appropriate data collection form. Information to be collected includes event description, date of onset, investigator assessment of severity, relationship to study product, date of resolution of the event, and outcome. Investigators will report promptly to the Sponsor any serious adverse event regarded as possibly, probably or definitely related to any study intervention.

AEs will be graded for severity and relationship to study product and followed to adequate resolution or stabilization, for participants enrolled in the study. After termination of the study, participants will be referred to appropriate care for follow-up of ongoing AEs. Any AE that is associated with the experimental vaccine will be followed to resolution, or stabilization if there is the expectation that it will remain chronic.

The Sponsor will notify, in writing, participating investigators of any adverse experience associated with the use of the PfSPZ Vaccine or PfSPZ-CVac that is both serious and unexpected and occurs in other trials of these products, and any finding from tests in laboratory animals that suggests a significant risk for human participants.

7.2.8.2 Serious adverse events

Any AE that meets a protocol-defined serious criterion will be reported by the PI or delegated study team member to the RM and the Sponsor within 24 hours of the investigator being aware of the SAE in an initial SAE report. All SAE reports and/or all SAE participants-

related safety information will be sent to Sanaria (the Sponsor) and addressed

SAEs will only be reported by the Sponsor to the US FDA and to the Indonesian regulatory authority (BPOM) in accordance with the reporting requirements and timelines for possibly, probably or definitely related SAEs. All SAEs will be reported by the Sponsor to the US FDA and by the PI (Sponsor's designee) to the Indonesian regulatory authority (BPOM) in the annual (or for BPOM, bi-annual) report. The Sponsor will notify the SMC chair of any SAEs reported as possibly, probably or definitely related to any study intervention.

A written follow-up report of the SAE by the PI or delegated study team member will be provided to the Sponsor no later than 5 working days from the original notification. Other supporting documentation of the event may be requested by Sanaria to meet its reporting obligations to the US FDA, BPOM and the SMC. The severity and causality of the event will be discussed between the PI and Sponsor and a consensus agreed upon prior to submission of that event to any ethical or regulatory body.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the Sponsor as outlined above. With the Sponsor's concurrence, the PI will be delegated to also report to BPOM. In addition, the investigator must also report SAEs to the ethics committee/IRB. Any related (possibly, probably, definitely) AE that meets a protocol-defined serious criterion with the concurrence of the Sponsor must be submitted:

- Within 48 hours to the Ethics Committee, Faculty of Medicine, Universitas Indonesia or as per their reporting requirements;
- Within 7 calendar days to the Indonesian food and drug regulatory agency, (BPOM) if it is a life threatening event or death (follow up report to be submitted within the next 8 days) or within 15 calendar days for other events or any other timeline as per their reporting requirements;
- To the US FDA as per their reporting requirements (to be submitted by Sanaria).

For other AEs, this information may be submitted:

- In the annual summary to the Ethics Committee, Faculty of Medicine, Universitas Indonesia
- In the annual summary to the Oxford Tropical Research Ethics Committee, University of Oxford; and
- In the bi-annual summary to BPOM
- In the annual summary to US FDA.

7.2.8.3 Serious and unexpected suspected adverse reactions (SUSAR)

A serious and unexpected suspected adverse reaction is a serious adverse reaction (SAR of which the nature or severity is not consistent with the applicable product information (e.g. Investigator's Brochure for an Investigational New Drug (IND), Investigational Medicinal Product (IMP) or Summary of Product Characteristics (SPC) for an authorized medicinal product), and for which there is a reasonable possibility that the investigational product (PfSPZ Vaccine or PfSPZ Challenge) caused the AE. In addition, the PI will report any SUSARs related to antimalarial chemoprophylaxis to the Sponsor

and local ethical and regulatory bodies within 15 days. The Sponsor will be responsible for reporting the SUSAR to the SMC and US FDA and notifying PIs of other clinical trials of PfSPZ Vaccine and PfSPZ-CVac or similar products. In turn, the Sponsor will inform the PI of this trial of any SUSARs that have arisen in other clinical trials of PfSPZ Vaccine and PfSPZ-CVac or similar products that could have consequences for the safety of the participants involved in this clinical trial.

The Sponsor will be responsible for reporting SUSARs to the SMC and US FDA; the PI, will report these to the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Oxford Tropical Research Ethics Committee and on behalf of the Sponsor to BPOM.

7.2.9 Type and duration of follow-up of participants after adverse events

Investigators will follow up participants with AEs and SAEs to adequate resolution or stabilization. Outcome will be assessed as Recovered/Resolved, Recovered/Resolved with Sequelae, Recovering/Resolving, Not recovered/Not resolved, or Fatal.

7.2.10 Pausing rules and safety hold

Rationale: As discussed in section 1.2.2, the safety and tolerability of PfSPZ Vaccine and PfSPZ-CVac manufactured by Sanaria have been very good. Nevertheless, important side effects not previously identified could emerge in this clinical trial, and for this reason research participants will be closely monitored. The study will have a Research Monitor (RM) to advise the PI, and an independent SMC to provide the Sponsor and clinical team with immediate external review and recommendations. The RM *and* the SMC chair will be notified of all related (possibly, probably or definitely) SAEs and also if the frequency of grade 3 (severe) adverse events exceeds 15% in either the PfSPZ Vaccine or the PfSPZ-CVac group, once a minimum of 20 participants has been enrolled into that group (the RM notified by the PI, the SMC chair by the Sponsor medical officer), and their counsel will be sought. Because the immunization window prior to deployment is extremely tight, and a clinical hold imposed on study progress due to AEs could essentially prevent successful immunization and compromise the scientific integrity of the trial, the pausing rules are structured for very rapid review of events to ensure the safety of the participants. Those entities with oversight are requested to understand that unjustified delays could result in trial cancellation based on the very restricted time window available for immunization prior to deployment. If a sequence of events unfolds where review and recommendation by IRBs and regulatory authorities is required (see below), these entities are requested to act within one week of the request.

Pausing rules and safety hold: In this study, “pausing” refers to an initial 24-hour suspension of immunization of the affected product(s) only, PfSPZ Vaccine and/or PfSPZ-CVac, to allow deliberations among the four principal clinicians with trial oversight: the PI, the RM, the Sponsor medical officer, and the chair of the SMC. This council of four clinicians may decide to resume the affected trial activities after the 24-hour suspension, but must review all available data and provide a written rationale for their decision to resume. Review from another oversight body such as IRBs and regulatory bodies will not be required to resume; however, IRBs and regulatory bodies will be informed of this decision in a timely manner. The criteria that result in a pause are specified below.

A second category is “extended pause,” which means that the aforementioned “pausing” will extend beyond 24 hours for up to three additional calendar days, to allow a formal meeting of the full

SMC. The PI, RM, Sponsor and SMC chair each individually have the authority to extend a pause beyond 24 hours in this fashion and request a full SMC meeting. In addition, there are criteria specified below that directly require a formal meeting of the full SMC.

After holding the meeting, the SMC may provide one of three general recommendations (plus any specific recommendations it may have):

1. Resumption of the affected trial activities, in which case immunizations may resume if the PI, RM, Sponsor and SMC agree. This can happen without the requirement for review from IRBs and regulatory bodies; however, IRBs and regulatory bodies will be informed of this decision in a timely manner.
2. Extending the pause further to allow the collection of additional essential (e.g., diagnostic) data and/or consultation with outside experts. Once the SMC is satisfied that it is safe to resume the affected trial activities, these may resume if the PI, RM, Sponsor and SMC agree. This can happen without the requirement for review from IRBs and regulatory bodies; however, IRBs and regulatory bodies will be informed of this decision in a timely manner.
3. Imposing a “safety hold” on the affected trial activities. This means the SMC recommends that IRBs and regulatory bodies should be informed of the event(s) and provided a chance for review prior to resuming the affected trial activities. If a safety hold is recommended by the SMC and imposed by the Sponsor, the IRBs and regulatory authorities will be notified as soon as possible of the hold going into effect. After the IRBs and the regulatory authorities are notified, they will have one week (seven calendar days) from the notification date to respond if they do not agree with a decision (and/or recommendations) to resume the study. The study will resume on the 8th calendar day after the last notification was received by an oversight body (IRB or regulatory authority), if no objections to study resumption are received by this time.

This tiered structure places an important responsibility on the shoulders of the SMC as an entity capable of rapid response times (as shown historically in Sanaria-sponsored studies). The SMC charter will specifically assure the independence from conflict of interest of the SMC members, including the RM, so that the SMC will act with safety as the priority. As described above, the SMC has the authority to recommend full review by IRBs and regulatory authorities. The IRBs and regulatory authorities have 7 calendar days to provide any feedback that they may have.

Criteria (refer also to Table 16. Below):

- A. The following criteria will be used as rules to pause the study:
 1. One or more participants experiences an SAE at any time after immunization that is determined to be possibly related to PfSPZ Vaccine or PfSPZ Challenge. This includes the transient parasitemia caused by PfSPZ Challenge but excludes CQ as the potentially causative agent.
 2. One or more participants experiences an SAE more than 14 days after immunization that is determined to be probably related to PfSPZ Vaccine or PfSPZ Challenge. This excludes CQ as the potentially causative agent.

3. One or more participants experience an SAE that is determined to be possibly related to CQ *and* is unexpected¹.
 4. 15% or more of at least 20 participant(s) experience the same Grade 3 local or systemic adverse event (solicited or unsolicited), including grade 3 or 4 laboratory abnormalities, in the +7 day post-vaccination follow-up period for PfSPZ Vaccine *or* the +14 day post-vaccination follow-up period for PfSPZ Challenge, that is deemed possibly, probably or definitely related to the study vaccine. This is 15% of the participants receiving the product in question (PfSPZ Vaccine, or PfSPZ Challenge).
- B. The following criteria will be used as rules to directly require a full SMC meeting:
1. One or more participants experiences an SAE within 14 days of immunization or less, that is determined to be probably related to PfSPZ Vaccine or PfSPZ Challenge, or an SAE at any time after immunization that is determined to be definitely related to PfSPZ Vaccine or PfSPZ Challenge.
 2. One or more participants experiences an unexpected SAE deemed to be probably or definitely related to CQ *and* is unexpected.
 3. 15% or more participant(s) experience the same Grade 3 local or systemic adverse event (solicited or unsolicited), including grade 3 or 4 laboratory abnormalities, in the 7-day post-vaccination follow-up period for PfSPZ Vaccine and the 12-day post-vaccination follow-up period for the first or second injections with PfSPZ Challenge (14 days after the third injection) deemed probably or definitely related to the study vaccine.

Table 16. Summary of Criteria:

Action	Event	Relationship	Timing
pause	SAE	possible	any
	SAE	probable	> 14 days
	SAE CQ (unexpected)	possible	any
	Severe AE in 15% of vaccine recipients	possible, probable, or definite in combination	≤ 7 days*
extended pause	SAE	probable	≤ 14 days
	SAE	definite	any
	SAE CQ (unexpected)	probable or definite	any
	Severe AE in 15% of vaccine recipients	probable or definite in combination	≤ 7 days*

* For PfSPZ Challenge, 14 days

¹ If an expected adverse reaction to CQ occurs that is serious or otherwise concerning, including encephalopathy, anxiety, psychosis, weakness, disturbances of vision, retinal changes, hearing loss, hemolysis, renal dysfunction, bone marrow toxicity, skin eruptions, cardiotoxicity, allergic reaction, etc., CQ administration will be stopped for that participant but will not lead to pausing the study.

Note: The unblinded pharmacist must confirm that the SAE or the severe AEs described above have occurred in study participants receiving active product. If the study participant has received placebo, the event(s) will be reported but without pausing the study. The pharmacist will have the treatment assignment list and will inform the PI whether study product was administered so as to minimize the amount of unblinding information provided. For example, if 5 participants must have received study product to meet the 15% criterion, the pharmacist will inform the PI that “criteria have been met” or “criteria have not been met,” but will not provide an unblinded list of individual study participants. In the extraordinary event of participants receiving normal saline placebo experiencing an SAE or AEs as detailed above, that is deemed possibly, probably or definitely related to normal saline, the team and oversight bodies will consider the improbable possibility of a problem with normal saline placebo formulation.

Authority to impose a pause or safety hold: Any oversight body, including the Sponsor, an IRB or regulatory authority, may pause or terminate the study for any appropriate reason, including issues with IP quarantine, research permissions, the safety of research personnel, adverse events in this trial or other trials of Sanaria products, etc. To resume execution of the trial, written permission of the entity imposing the safety hold and of the Sponsor will be required.

Continuation of chloroquine: If PfSPZ Challenge immunizations are placed on hold, CQ administration may continue on a weekly basis until PfSPZ Challenge injections are resumed.

Reporting: Any events leading to study pause, extended pause or safety hold will be reported to cognizant IRBs and regulatory agencies including the US FDA and BPOM according to their specified timelines and/or as described above. Reports to IRBs and in-country regulatory authorities (delegated by the Sponsor) will be made by the PI after review of submissions packages by the Sponsor. Reports to the SMC and US FDA will be made by the Sponsor. The PI is not to directly contact the SMC unless asked to do so in writing by the Sponsor.

7.2.11 Safety oversight

7.2.11.1 Safety Monitoring Committee (SMC)

This clinical trial will utilize a SMC, which is an independent group of experts that advises the Sponsor and through the Sponsor, the study investigators. The SMC will be an independent multidisciplinary group whose members are not involved with the trial in any other way, or have direct or indirect competing interests that could impact on the trial. The SMC will be established by the Sponsor and all communication to the SMC will be through the Sponsor, except at meetings during which the PI will participate. The primary responsibilities of the SMC will be to 1) review and evaluate study data for participant safety, study conduct and progress, 2) make recommendations to the Sponsor concerning the continuation, modification or termination of the trial and 3) provide advice regarding the medical care of individual participants and whether or not they should continue participating in the trial. The SMC will be composed of a quorum at least two voting members. Procedures for SMC data reviews will be defined in a SMC charter

that will include membership, responsibilities, definition of a quorum and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The Sponsor or the SMC may request ad hoc meetings according to protocol criteria or if there are concerns that arise during the study. After each assessment, the SMC will provide recommendations as requested by the Sponsor.

The following SMC teleconferences will be scheduled:

- An introductory meeting prior to enrollment of participants
- One data review meeting or email exchange during the course of immunization after approximately one quarter to one third of the participants in the PfSPZ-CVac group have been immunized and their clinical responses to transient parasitemia observed (immunizations will not pause to await the outcome of this meeting).
- One data review meeting after all the participants have been immunized and the initial surveillance period is underway.
- A close-out meeting at the end of the study after database lock.

In addition, ad hoc teleconferences will be scheduled:

- If a pausing rule is met immunizations will be paused until the SMC recommendation is received and the Sponsor makes a determination regarding continuation of immunizations.
- To discuss any issue of safety raised by an investigator, the MM, the Sponsor, or a member of the SMC.

Data will be provided to the SMC as safety reports containing blinded data in a standard summary format. If unblinded data are requested by the SMC, they will be provided by the data management company and presented and discussed in an optional closed session of the SMC. The questions the SMC is to address during any given meeting will be provided prior to the meeting by the Sponsor, so that the SMC may develop appropriate recommendations.

7.2.11.2 Research Monitor (RM)

The Research Monitor (RM) (= medical monitor = safety monitor) will be a physician with relevant expertise whose primary responsibility will be to provide the PI and the Sponsor with an independent clinical safety assessment in a timely fashion. Participation is for the duration of the study and is a wholly voluntary position without financial compensation.

The RM will:

- Have the ability to readily access study participant records in real time by any reliable means.
- Not be in a direct supervisory relationship with the investigator.
- Not have any direct involvement in the conduct of the study.
- Sign a COI certification at the time they are asked to participate and provide updates to this information as needed.
- Receive reports of Serious Adverse Events (SAEs) or Unanticipated Events (UA) from the site investigator and will be notified by email when Sanaria is notified of the SAE or UA.

- Evaluate SAEs and Uas and report their clinical assessment to the PI and Sponsor in a timely manner.
- Communicate with the investigator at the participating site as needed.
- Review additional safety related events at the request of the Sponsor.
- Provide additional information to the Sponsor and/or the SMC by teleconference as requested.
- Have the authority to pause the study in progress,
- Have the authority to remove individual human participants from the study.
- Take whatever steps are necessary to protect the safety and well-being of human participants until the SMC and IRB can assess the PI's or monitor's report.
- Promptly report his/her observations and findings to the Sponsor, and to the IRB or other designated official if not already done by the PI.
- Promptly report his/her observations to the funder's Human Research Protections Office (HRPO) if not already done by the Sponsor or PI.

8 CLINICAL MONITORING

Study site clinical monitoring will be conducted by the clinical monitoring contractor to ensure that good clinical practice standards and regulatory guidelines are being followed, and specifically that the protocol is being followed. A pre-trial clinical monitoring site initiation visit will be made to the study site, including the clinical laboratory. All records will be made available to monitors, including regulatory files, CRFs and source documents, QA/QC documentation, SOPs, etc. Additional study site visits will be made during the course of the trial and at the end of the surveillance period.

In conjunction with the clinical monitoring contractor designated by the Sponsor, a detailed monitoring plan will be developed. This document describes who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted. Specifically, the monitoring plan will include the number of participant charts to be reviewed, which/what proportion of data fields and what will be monitored, who will be responsible for conducting the monitoring visits, and who will be responsible for ensuring that monitoring findings are addressed. The Clinical Monitoring Plan will be written by the clinical monitoring contractor and the Sponsor.

9 STATISTICAL CONSIDERATIONS

Data entry will be performed onsite and in the data management unit in Jakarta. StatPlus will perform data analysis and report primary and secondary endpoint results. In collaboration with the OUCRU ID team, StatPlus will review and finalize a Statistical Analysis Plan. This contractor will be bound by strict rules of confidentiality and will have no authority to disseminate any aspect of this trial beyond designated authorized parties.

9.1 Study Hypotheses

Primary Endpoints

Safety: The primary safety endpoints are (a) the proportion of vaccinees compared to controls experiencing solicited AEs within 7 days (PfSPZ Vaccine) or 14 days (PfSPZ-CVac) of each administration of investigational product (IP), (b) the proportion of vaccinees compared to controls experiencing SAEs during the trial deemed related to IP, and (c) proportion of participants experiencing unsolicited AEs occurring within 14 days of each administration of IP deemed related to IP. There is no formal hypothesis test associated with this primary safety endpoint. Rather, the proportions of participants experiencing AEs will be compared between treatment groups using frequencies, percentages, and descriptive 95% confidence intervals (CIs).

Efficacy: the primary efficacy endpoint is VE, computed as one minus the estimated hazard ratio (HR) for first Pf *malaria* case detected by blood smear. Blood smears are deemed positive if at least one unambiguous asexual blood stage parasite is identified by two independent microscopists after each examining 0.50 µL of blood. The formal hypothesis associated with this single primary efficacy endpoint is a superiority test of

$$H_0: VE = 0 \text{ versus } H_A: VE < > 0$$

(equivalently, $H_0: HR = 1$ vs. $H_A: HR < > 1$). Each participant's entry into the risk set will begin 14 days after arrival in the malaria-endemic area. Participants who do not experience a Pf *malaria* case by the end of deployment will be censored from the primary analysis on that date. The primary efficacy hypothesis test will be conducted at the two-sided $\alpha=0.05$ significance level, restricted to participants in the ITT population (defined below).

9.2 Sample Size Considerations

Vaccine efficacy (VE) will be estimated separately for PfSPZ Vaccine and PfSPZ-CVac by the following two methods:

1. The *hazard rate method* ($VE = 1 - HR$) where HR is the unadjusted hazard ratio from the Cox proportional hazards model.
2. The *proportional method*: $VE = 1 - RR$, where RR is the risk ratio. Here, $RR = \frac{p_v}{p_c}$, where p_v is the proportion of vaccinees infected within 6 months of follow-up, and p_c the analogous proportion for controls.

The hazard rate method (survival/time to event analysis) will be considered the primary method for estimating VE, and VE by PfSPZ Vaccine will be considered the primary VE analysis and VE by PfSPZ-CVac the secondary. Past studies have indicated an incidence of 1.5 and 3 infections/person-year for both Pf and Pv [59-64]. This translates to a 6-month cumulative incidence of 75 - 100%. The 6-month cumulative incidence is defined as the proportion of control participants with at least one malaria infection within 6 months of follow-up. In estimating sample size, we assume a more conservative estimate of 50%, but also perform estimates for 12.5% and 25%.

Power was calculated by simulation, assuming 10% dropout, and assuming that the true efficacy by the proportional method is equal to 0.56. 10,000 simulations were performed.

In this analysis two approaches for hypothesis testing were considered, for the proportional: Fisher's exact test, and modified Poisson regression. The latter is more powerful, and also would allow us to adjust for covariates as a secondary analysis, which can add precision. The log rank test (equivalent to score intervals for the Cox proportional hazards model) is used to compare the hazards. Power is estimated as the proportion of simulations in which the null hypothesis is rejected. Simulations were repeated for various sample sizes to obtain the sample size needed for 80% power by the survival analysis method. If the incidence is as expected in the controls (0.25) and VE is 56% then the sample size of 124 vaccines and 124 controls is adequate to assess VE by all methods. However, the primary will be survival analysis ($\alpha = 0.05$).

Table 17. Incidence, power, and sample size.

6-month incidence	Sample size per group	Sample size after dropout	Power (Fisher's)	Power (Poisson)	Power (survival)
0.50	49	44	0.74	0.78	0.80
0.25	125	112	0.75	0.78	0.80
0.125	286	257	0.76	0.79	0.80

The assumed attack rate of 0.25 Pf infections per soldier per six months is conservative. Recently battalions stationed in Papua have experienced higher attack rates. Therefore, if it proves difficult to reach the target sample size during enrollment, a total sample size of 340 participants will be deemed adequate.

9.3 Final Analysis Plan

A finalized statistical analysis plan will be agreed upon by the investigators and Sanaria (and its StatPlus contractor) before locking the database for final analysis. Analysis of all primary endpoints and the first secondary endpoint will be conducted on data and samples collected through the end of the Exposure phase of the study. This analysis will be used for decision-making related to the product clinical development plan and will be available for public presentation after the primary data is locked. The study will continue for additional surveillance and immunogenicity assessment until the end of the Post-Exposure period, which will be 24-28 weeks later. This additional information will be appended to the study report. It is anticipated that the results of this study will be presented to the scientific community

via oral presentations at meetings and written publications in scientific journals. The data to be presented and the authorship will be discussed between partners before any official communication.

The official report of the primary analysis will be written by the study investigators and the statistical consultants with support from Sanaria, reviewed by all partners, and submitted through appropriate channels for approval. This report will contain detailed information about the participants, their tolerance of the vaccines, their side effects and laboratory abnormalities, as well as their overall immune responses to vaccination.

9.3.1 Analysis of demographics

Demographic characteristics (age, gender, and neighborhood of residence) of each study group will be tabulated. The mean age (plus range and standard deviation) of the enrolled participants, as a whole and per group will be tabulated and statistically analyzed with respect to homogeneity of these between randomized groups.

9.3.2 Analysis of safety

The overall proportion of participants with at least one solicited local reactogenicity event and the proportion with at least one solicited systemic reactogenicity event during the defined surveillance periods after initiation of vaccination will be tabulated. Likewise, the proportion of participants with at least one unsolicited local AE and the proportion with at least one unsolicited general AE during the 12 to 14-day surveillance period after vaccination will be tabulated.

The incidence, intensity and relationship of individual solicited AEs to the vaccine over the surveillance periods will be calculated per group and vaccine administration. The incidence, intensity and relationship of individual unsolicited AE, classified using MedDRA System Organ Classes and Preferred Terms, reported after vaccination will be tabulated per group and vaccine administration.

SAEs will be described. Comparisons between study groups of incidence of events, including solicited local and systemic reactogenicity events will be made based on two-sided Fisher exact tests. Analysis of safety during the initial surveillance period will consist of comparison of incidence of SAEs, as well as hemoglobin, white blood cell, platelet, creatinine, and ALT levels.

9.3.3 Analysis of clinical laboratory parameters

Hematological (CBC) and biochemical (ALT, creatinine) laboratory parameters will be measured at specific time points after each vaccination. Clinically relevant abnormal values based on reference intervals determined in a similar population will be tabulated and a trend analysis performed if deemed necessary. Laboratory abnormalities will be graded on a scale of 1-3 as defined in **Tables 14 and 15: Laboratory Toxicity Grading**.

9.3.4 Analysis of humoral immunogenicity

The immunogenicity endpoints-- antibodies against PfCSP and potentially other Pf proteins, will be assessed in several ways. A series of graphs will display immunologic responses. There will be one primary method used for assessing antibody response: ELISA (spectroscopically assessed enzyme-linked immunosorbent assay) using a PfCSP recombinant protein as antigen. At a minimum, the PfCSP ELISA will be used to determine relative levels of antibodies in sera taken before vaccination and 12-

14 days after the last dose in all participants. Levels will be examined to assess possible association with protection. An aIFA (microscopically assessed immuno-fluorescent assay) using PfSPZ and potentially other parasite stages and ELISA using other Pf proteins and inhibition of sporozoite invasion (aISI) assay may be done based on the results of the first set of assays and availability of funding. Corresponding summary statistics will show medians or means and standard deviations, and an effort will be made to determine a threshold for protection. Cox proportional hazards modeling will be used to estimate the relationship between antibody levels and the time to first parasitemia. Separate models will be fit for the time to parasitemia measured by qPCR and time to parasitemia measured by microscopy. The antibody assays and analyses will be conducted essentially as described [13].

9.3.5 Analysis of CMI responses

For other trials of PfSPZ Vaccine and PfSPZ-CVac, cellular immune responses against PfSPZ and Pf asexual erythrocytic stages (AES) have been primarily assessed by flow cytometry using intracellular cytokine staining (ICS) [9, 10, 12, 13, 21]. Peripheral blood mononuclear cells (PBMCs) will be isolated and stored for such analyses. If done, they will be done using samples collected approximately +14 days after the first and third doses of PfSPZ-CVac and +14 days after the second and third doses of PfSPZ Vaccine. Specimens will also be collected at the end of the Exposure phase and at the end of the Post-Exposure phase (end of study). ICS by flow cytometry analysis will be dependent upon PBMC availability and additional funding to conduct. Correlations between continuous measures of immune response will be assessed using the standard Pearson as well as Spearman rank correlation coefficients, on \log_{10} transformed data when a logarithmic transformation results in a distribution more nearly normal than the distribution of untransformed values. Association between a continuous and a categorical measure will be assessed using t-tests or analysis of variance, or the analogous tests on ranks. Association between categorical measures will be assessed using chi-square or exact tests. Appropriate summary descriptive statistics (e.g., means and standard deviations or medians and ranges) will be presented. Effects of covariates (e.g., gender, ethnicity) will be assessed using regression models.

9.3.6 Analysis of efficacy against infection

Time-to-event analysis will be used to compare time until first clinical malaria case caused by Pf between the control and treatment groups. The Kaplan Meier method will be used to display survival curves for the two groups, and the log rank test will be performed to test whether the survival curves differ between treatment and control groups. The unadjusted hazard ratio will be presented along with its 95% confidence interval, calculated using the likelihood score method. As a secondary analysis, the hazard ratio will be computed adjusting for baseline characteristics using Cox proportional hazards modeling. Vaccine efficacy will be estimated as $1 - p_v/p_c$, where p_v and p_c are risks of at least one malaria infection in the 6-9 month Exposure period. If all participants are followed for the entire Exposure period, p_v and p_c will be estimated as simple proportions. However, if there is any loss to follow-up after the post-vaccination antimalarial treatment, p_v and/or p_c will be estimated by the Kaplan-Meier method. Two-sided 95% confidence intervals around the point estimates of VE will be calculated, using a likelihood score or Taylor series method. Vaccine and placebo recipients will also be compared with respect to the proportion of individuals experiencing at least one malaria episode after randomization (ITT analysis), the number of malaria infections occurring in each individual, and number of clinical

malaria events, will also be done. Asexual Pf parasite density, measured as area under the curve, will also be reported for vaccine and for placebo recipients.

9.3.7 Analysis of strain-specific efficacy and selection (optional activity)

Rationale: PfSPZ Vaccine and PfSPZ-CVac provided 100% VE against CHMI by a strain of Pf identical to the vaccine strain in malaria-naïve U.S. and German participants and semi-immune Tanzanian and Malian participants. It is hoped that this vaccine will provide similar high vaccine efficacy in Indonesian soldiers who are exposed to genetically diverse natural Pf infections. This initial test of these vaccines against natural challenge by genetically diverse parasites in Papua will afford a valuable first opportunity to develop and use novel genomic epidemiology methods and analytical strategies for assessing the molecular basis of whole-parasite vaccine protection [65]. These exploratory analyses may lead to fundamental advances in our understanding of pre-erythrocytic protective immunity, but more importantly this work represents the first steps toward determining whether a PfSPZ-based vaccine will protect against the extremely diverse malaria parasites found in Papua.

Approach: For vaccine and placebo groups, occurrence of infection with parasites with genotypes identical to the NF54 vaccine strain will be recorded. In addition to strict identity and non-identity with the vaccine strain, measures of genetic diversity between the NF54 vaccine strain and infections occurring in study participants will also be measured. Diversity will be measured using microsatellite maps, genome-wide single nucleotide polymorphism (SNP) typing platforms including a microarray that detects 33,000 Pf SNPs developed by the UMD/CVD Division of Malaria Research, and/or by next-generation genome sequencing. Methods for microsatellite typing are robust and well established at the UMD Center for Vaccine Development [66, 67]; methods for SNP typing and genome sequencing are also established in the UMD Division of Malaria Research in collaboration with the UMD Institute for Genome Sciences (C. Plowe, unpublished). Parasite DNA extracted from leukocyte-depleted blood collected during clinical episodes and follow-up visits will be participated to genome-wide genotyping and genome sequencing at the EIMB by staff trained at UMD and with support and training from UMD staff.

Analysis: Sieve analysis is a method for determining how VE varies according to genotype of the infecting pathogen, which has been used to measure strain-specific efficacy in HIV vaccine trials [68]. The “sieve” is the vaccine-induced immune barrier to infection. Sieve analysis involves identifying “holes” in the sieve, i.e. pathogen characteristics—such as DNA sequence divergence from the vaccine strain at a given locus—that allow it to pass through the barrier created by vaccine immunity. We used this method to measure strain-specific efficacy in a Phase 2 trial of a blood-stage malaria vaccine [69, 70]. The analysis will focus on parasite genomes in vaccinated and control individuals during the 6-9 month Exposure phase. We will estimate strain-specific odds ratios by calculating the odds of infection by a given allele in breakthrough infections in vaccinated individuals compared to the odds of infection by that strain in infected unvaccinated individuals. Vaccine relative risk ratios will be approximated by dividing the odds ratio for a given strain by the odds ratio for the vaccine strain [70]. We will perform this sieve analysis both genome-wide, but also focusing on candidate genes encoding pre-erythrocytic antigens that may contribute to whole PfSPZ vaccine induced VE such as PfCSP, PfLSA1 and PfSSP2/TRAP. “Strains” (or “variants”) will be defined both at the level of individual amino acids and at the gene level in ordered categories of genetic distance between the gene sequence of the breakthrough infections compared to that of the vaccine strain. Loci with inflated vaccine relative risk

ratios, after adjusting for multiple comparisons, will be considered to be under selection by the vaccine and thus represent the key antigens responsible for driving strain-specific protective efficacy. We will use a similar rationale on a genome-wide level to look for evidence of allele-specific efficacy and vaccine-induced selection in loci not determined *a priori* to be of interest. We will (i) identify regions of reduced polymorphism in parasites from breakthrough infections from vaccinated individuals compared to those sampled from infected unvaccinated individuals, and (ii) identify regions where average genetic distance to the vaccine strain is highest in parasites from breakthrough infections relative to the average observed for parasites sampled from infected unvaccinated individuals. We will use a sliding window approach, with optimal window and step size determined empirically from the data. Genes located in regions of interest will be carefully evaluated for function and expression profile.

Depending on the efficacy of PfSPZ Vaccine and PfSPZ-CVac, the number of breakthrough infections from this first trial of these vaccines in Papua may be too small to provide sufficient power for these genomic epidemiology analyses to detect statistically significant differences. Through the International PfSPZ Consortium (I-PfSPZ-C) we are forming a network of investigators around the world who plan to design and conduct trials to evaluate PfSPZ vaccines in malaria-exposed populations. Results from genomic analyses for this first trial will eventually be pooled with those of contemporaneous and subsequent trials to accomplish a larger sample size for these exploratory analyses.

9.3.8 Missing data

Every effort will be made to minimize missing data and collect all endpoints specified in this protocol. Participants who discontinue treatment will be followed after treatment discontinuation for collection of all scheduled safety data with their consent. No adjustments for missing safety data will be performed for the primary analysis. The efficacy objectives of the study are to compare time to parasitemia between treatment arms and to compare proportions infected at six months post-vaccination between treatment arms. If parasitemia measurements are missing due to dropout, the survival analysis proposed will naturally adjust for the missing response variable. Thus, no additional adjustment for missing data is needed. Survival analysis assumes that the relationship between treatment arm and parasitemia does not differ between participants who drop out and those who remain in the study. If dropout occurs, the validity of this assumption may be evaluated by comparing these participants on other relevant covariates and/or through sensitivity analysis.

9.4 Study Cohorts/Datasets to be Evaluated

9.4.1 Per protocol cohort

The 'Per Protocol Cohort' will include all eligible participants randomized to study vaccine or placebo, who received all assigned vaccinations with the study vaccine or placebo control within correct time windows, who received at least 80% of the injectate with each immunization, and who received the correct vaccine. Participants will be classified according to the treatment (vaccine or placebo) received. Participants found retrospectively to be qPCR positive for malaria on enrollment will be excluded from this cohort.

Added with Protocol Version 4.0. Due to the fact that all PfSPZ Vaccine / placebo immunizations were performed out of window (significantly delayed), a “modified per protocol” (mPP) cohort is defined that while requiring first and second immunizations to be within window, the third immunization may be delayed. All other criteria still apply.

9.4.2 Intent to treat cohort

The ‘Intention-to-Treat’ Cohort’ (ITT cohort) will include all participants randomized to the study vaccine or placebo, classified according to the randomization assignment. Participants must receive at least one immunization. Safety analyses will be done on this cohort.

Added with Protocol Version 4.0. Since the original protocol was written in 2017, Sanaria has started to perform primary analyses on a “modified intention to treat (mITT) population defined as having received all three vaccinations, even if not necessarily within window or even if the full volume of injectate was not administered. The use of mITT for the primary analysis applies to Phase 1 and Phase 2 trials. So that the results of the IDSPZV1 trial will be comparable to other trials, the same convention is adopted here. Consequently, the mITT cohort will be the primary analysis cohort. It will include all participants who received three immunizations, even if some immunizations were out of window or less than 80% of the injectate was administered. Like the PP cohort, any participants diagnosed with parasitemia or clinical malaria at any time during the immunization period will be excluded. Participants will be classified according to the treatment (vaccine or placebo) received.

9.4.3 Safety cohort

The ‘Safety Cohort’ will consist of all participants who have received at least one dose of study vaccine or placebo and for whom any data on safety are available. Participants will be classified according to the treatment received. The primary safety analysis will be done on this cohort.

The presentation of safety data will explore separately the adverse events among participants who received all vaccinations, among those who received only some and among those with protocol deviations related to receipt of study products.

9.4.4 Immunogenicity cohort

The ‘Immunogenicity Cohort’ will include all evaluable participants (i.e., those meeting all eligibility criteria, and who have received at least one vaccination with the study vaccine or placebo) for whom data concerning immunogenicity endpoint measures are available. This cohort will include participants for whom assay results are available for antibodies against at least one component antigen of the study vaccine antigen component after vaccination. Participants will be classified according to the treatment received. Immunogenicity analysis will be done using this cohort after the analysis done on the Per Protocol cohort, as appropriate.

10 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating study site will maintain appropriate medical and research records for this trial, in compliance with International Conference on Harmonisation (ICH) harmonised tripartite guideline E6(R2): Good Clinical Practice (GCP), section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participants' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. All stained blood smears collected in this trial will be carefully preserved and retrievably archived in a single secure location.

Electronic CRFs (eCRFs) will be supplied by StatPlus under contract to Sanaria, and an electronic remote data entry system will be used (Viedoc). Data collection forms derived from the eCRFs will be made available and maintained in individual participant charts for each participant, and will be maintained at the study site; when not in use all source documents will be stored in a secure location with limited access. All data collection forms will be filled out completely and by appropriate study personnel. These data collection forms will serve as source documents.

The study site will permit authorized representatives of Sanaria, their designees, and appropriate regulatory agencies 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. These representatives will be permitted access to all source data under strict rules of confidentiality agreed upon by all parties to this study protocol.

11 QUALITY CONTROL AND QUALITY ASSURANCE

The OUCRU ID team will develop a site quality management plan. They will then conduct routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The quality management plan will be located at OUCRU ID's central offices in Jakarta. The protocol-specific quality management plan will be reviewed by Sanaria's clinical and regulatory teams. It describes the study site's internal quality management activities including how the data will be evaluated for compliance with the protocol, which documents will be reviewed, and methods of training staff.

Standard operating procedures (SOPs) will be used at all clinical and laboratory sites. Routine monitoring will be performed according to ICH/GCP (E6) (e.g., data monitoring). Sponsor-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. The study site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by Sanaria, and inspection by local and regulatory authorities.

StatPlus and the study site data manager will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the study site for clarification/resolution.

12 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

12.1 Ethical Standard

Studies of Sanaria® PfSPZ Vaccine are conducted under appropriate Investigational New Drug (IND) applications filed with the US FDA. The study described in this protocol will be conducted according to current Good Clinical Practices (US 21 CFR Part 50-Protection of Human Participants and Part 56-Institutional Review Boards, US 45 CFR 46, 21 CFR 312, the Declaration of Helsinki, and the applicable rules and regulations of Indonesia).

The following institutional ethical review committees will consider this protocol for approval:

1. Ethics Committee of the Faculty of Medicine Universitas Indonesia (EC-FMUI), Jakarta, Indonesia
2. Oxford University Tropical Research Ethics Committee (OXTREC), Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

The trial will not proceed without formal approvals from both committees being stored in the formal file of this study held at OUCRU ID and FMUI's IOCRL facility in Jakarta, Indonesia, in addition to being registered with a trial number at www.clinicaltrials.gov.

12.2 Institutional Review Board Procedures

All post-approval amendments to this protocol will be submitted to REC-FMUI and OXTREC, as well as to Sanaria. No amendments will go into effect without written approval from each of these entities except when deemed necessary by the PI or site medical office (SMO) to eliminate immediate hazards to participants. Protocol deviations will also be reported to REC-FMUI and OXTREC in accordance with their respective policies.

The investigators will inform EC-FMUI, OXTREC, and Sanaria of the following:

- All subsequent protocol amendments, informed consent form changes or revisions of other documents originally submitted for review
- SAEs or unusual/unexpected AEs occurring during the study, within required timeframes
- New information, including any provided by Sanaria from other research activities, that may affect adversely the safety of the participants or the conduct of the study
- An annual update and/or continuing review reports, as required
- A final report including SAE outcomes will be provided when the study has been completed

12.3 Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out.

Information will be given in both oral and written form whenever possible. The consent documents will embody the elements of informed consent as described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable host country regulations. The oral consent process will be consistent with 45CFR46, 46.117, 21CFR50.27 and ICH E6 (R2) section 4.8. Independent witnesses will be used to attest that illiterate potential participants have understood the contents of the informed consent document.

12.4 Screening and Study Informed Consent

A prolonged and deliberate work plan of study familiarization and institutional assent to participate by the military chain-of-command of the study battalion will occur long before soldiers are invited to participate as study participants in this trial. The benefits and risks of the procedures of this trial were first presented to the senior-most health officers of the Indonesian Army who then approached line military senior commanders for their approval for engaging the combined civilian-military medical research team in this endeavor. At all levels, commanders consider potential benefits to the health of Indonesian soldiers and the capacity for engagement and participation to potentially interfere with their primary duties and responsibilities as a functioning infantry unit with a broad security mission to accomplish. With the assent of senior officers of central Army command, the research team will then approach senior regional Army commanders for approval to finally engage the commander of the specific battalion of study at their home base. That commander has the responsibility for the operational integrity and ability of his battalion, along with the safety and welfare of every soldier assigned to it. His assent then finally allows the research team access to the soldiers of that battalion.

The team first approaches the officers of the battalion, explaining the purpose, processes, risks and benefits of the trial in a private meeting room. Questions or concerns are addressed. The research team then emphasizes the extreme importance of voluntary consent to participate to the protection of the integrity of the trial. The intrinsically coercive character of military cultures everywhere must be directly addressed and participation in the trial acknowledged as a reserve where chain-of-command wishes or orders may not be imposed. There is mutual agreement that soldiers may not be ordered to participate or offered special reward or recognition for doing so. Likewise, soldiers who decline consent may not be punished or ostracized in any direct or indirect fashion. Instructions from the chain-of-command at the highest level explicitly prohibit the ordering of any soldier to participate as a study participant.

The next step is for the research team to approach the rank and file of the battalion in groups for planned sessions aimed at study familiarization (purpose, processes, risks, benefits), along with expressing the same philosophy on the importance of voluntary consent to participate as a human participant of medical research. We document the content and attendance of all of these sessions with signature affirmations of all present. After inviting questions and discussion, the session ends and the soldiers who attended are finally invited to come to the study center on the base for a participation screening appointment. This engagement is conducted according to an SOP on file in the OUCRU clinical trial admin office. With command permission, the sessions may also be videotaped and stored in the clinical trial file.

Upon arrival at the study center, soldiers are invited into a private room with a member of research team and an impartial witness. The study is again briefly explained verbally, and the purpose of the informed consent form is explained. The form is then read to the soldier in Indonesian language. He is offered the form and the opportunity to read it for himself, including the offer to take it with him and come back when he has decided about signing it or not. A soldier expressing a willingness to offer consent is invited to sign the form, along with an impartial witness and the research team member conducting the screening. The impartial witnesses will likely be personnel assigned to the base but not scheduled to deploy with the battalion (and therefore not eligible for participation).

Soldiers signing the consent form are registered in a screening logbook that utilizes their study number. The study participant candidate then progresses (carrying his study chart) to clinical and

laboratory screening in the study laboratory on the military base. Source documents, like ECG readouts and laboratory findings are labeled with a sticker and placed into the participant chart. After collecting demographic details, vital signs, and a medical history and partial physical examination, the study physician determines suitability for proceeding to laboratory screening. Eligible participants are referred for venous blood extraction in the study laboratory, presenting their charts to study laboratory staff. Venous blood is labeled with a sticker matched to the study ID number and proceeds for screening/baseline blood testing as prescribed.

After the laboratory findings are reported and entered into the CRF, the SMO or another physician delegated this responsibility, will inspect all findings therein and reach a decision regarding the eligibility of the participant to proceed for first vaccination. The SMO thus generates a list of participants screened as eligible for vaccination. Names on that list are contacted to return to the study center for enrollment. Participant will be randomized and the assignment into PfSPZ Vaccine or PfSPZ-CVac groups will be revealed (that assignment is open label), while the assignment to vaccine or placebo will not be revealed. The participant will then be scheduled to return for his first vaccination. If enrolled into PfSPZ-CVac group, participant is administered the first loading of CQ chemoprophylaxis approximately two days prior to first immunization. If the participant is enrolled into the PfSPZ Vaccine group, first vaccination will be scheduled roughly 4 weeks into the future, since the immunization regimen for PfSPZ Vaccine is one month shorter than for PfSPZ-CVac.

On vaccination day, participant will be asked to gather at the front of the study center for bus transport to the study vaccination site ideally located off base and therefore accessible by foreign nationals representing the Sponsor who will directly oversee and supervise at least the first 100 vaccinations. However, if vaccination is done on a military base, and direct Sponsor oversight is not possible, that will be deemed acceptable.

Any screened participant who is excluded from that invitation to be vaccinated will be invited to the clinic to be informed of the medical basis of that decision. That participant's ID number is recorded as excluded on clinical grounds, those being recorded in his CRF. Another soldier may be enrolled with another study ID number to replace him in striving to reach the enrolment target of 372 participants.

Any screened participant assigned a study ID number who decides to withdraw consent to participate before being vaccinated is recorded as such a withdrawal and another soldier may be enrolled to replace him in the target of 372 participants. Any participant thus receiving first vaccination is considered entered into the intention to treat population (see below). Discontinuation for any reason constitutes a withdrawal without replacement by a new participant.

12.5 Compensation

To compensate participant soldiers for their time and trouble in dealing with the research team, we will offer an incentive of IDR 100,000 (about USD 7.50) for each essential visit to the study clinic or vaccination facility. During deployment to Eastern Indonesia participant will not be offered similar compensation if study team visit the participant at his post rather than have the participant come to the clinic. However, transportation reimbursement of IDR 50,000 will be offered to soldiers reporting to the study clinic acutely ill. Compensation may or may not be offered during the six month post-redeployment surveillance period, depending on resources available.

12.6 Exclusion of Women, Minorities, and Children (Special Populations)

The conduct of this trial in a line infantry battalion of the Army of the Republic of Indonesia excludes women and children from participation. The majority of the study population will likely be ethnic Javanese, Balinese or Sumatran men. The Indonesian Army does not include women in deployed infantry units.

12.7 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the Sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study clinical monitor or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. These records will be de-identified prior to inspection and will be labeled only with the unique participant ID number. Additionally, representatives of the FDA and Indonesian regulatory authority (BPOM) may review your personal and medical information (which in this case, may not be de-identified). The study site will permit access to such records.

Participants will be assigned this unique participant ID number. All results will be keyed to this number. Study records will only be available to staff members and will be kept locked at the study site conforming to the investigators' SOPs. Following the conclusion of the study, all records will be maintained on site for a minimum of two years, after which they will be stored long-term at data storage facilities in Jakarta for 10 years. All records will be retained in locked metal boxes for at least two years after a marketing application is approved for PfSPZ Vaccine or PfSPZ-CVac; or, if an application is not approved, until two years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. After either of these conditions has been met with permission of Sanaria, records will be destroyed.

12.8 Study Discontinuation

The study may be discontinued at the discretion of the Sponsor, the PI, the regulatory authorities (FDA and BPOM), and the ECs/IRBs. In the event that the study is discontinued before completion, all participants who received study product will be asked to follow up with the study team for debriefing. Study team personnel will be available for questions or follow-up should evaluation be needed. When the study ends, participants will be advised to resume normal health-seeking behaviors with the Army health facilities. AEs that are ongoing at the time of study discontinuation will be followed by study staff to resolution, or, if a chronic condition has developed, until the end of the study follow-up period. As PfSPZ Vaccine and PfSPZ-CVac are not licensed products, they will not be offered to placebo recipients at the time of study discontinuation.

12.9 Future Use of Stored Specimens

If residual nucleic acids, sera and cells are available following the serological, cellular, and genomic assays described in this protocol, additional immunological and *in vitro* studies may be performed on those samples for which permission was expressly granted for preserving samples for future studies at the time of informed consent at study enrollment. These assays may include antibody epitope mapping, determination of response to other allelic forms of key parasite antigens, the ability of participant sera to interfere with *in vitro* parasite growth or invasion in an antigen-specific fashion, differential recognition of Pf or Pv proteins pre- and post-vaccination and in protected and non-protected individuals, sequence and expression analyses of parasite nucleic acids, and production of monoclonal antibodies. Additional research questions to be asked for cells include antigen-specific cytokine induction as measured by ELISpot, flow cytometry, or both, or additional analysis to determine specificity of lymphoproliferation responses. These immunological studies will be limited to immune responses to malaria and mosquito antigens and intrinsic immune responses and gene expression unless specific permission for additional studies is obtained from the relevant IRBs.

Samples from participants who did not grant permission to preserve samples will be discarded after the primary and secondary analyses described in this protocol have been completed. Study participants will have the right to withdraw their permission for further use of their samples at any time during and after the study. There will be no limitations on future use of cultured parasite lineages originally derived from clinical samples but which no longer have any human materials present.

No biological material collected in this trial – parasitic or human – will be transferred into the custody of persons beyond the borders of the Republic of Indonesia without an official Material Transfer Agreement (MTA) approved by the Ministry of Health.

Excess specimens for possible future use will be kept at the OUCRU ID/FMUI IOCRL clinical repository. These samples are the property and responsibility of the *PRBME – BRIN* and their Indonesian Army counterparts and research partners, and any research use of them may be done only with the expressed written permission of a responsible and authorized *PRBME – BRIN* officer representing that institution and the Indonesian Army.

13 DATA HANDLING AND RECORD KEEPING

The principal and responsible investigators are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. **DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.**

Data collection forms will serve as the repository for primary source documents. Only information that cannot be collected initially onto the data collection forms (namely, laboratory test results and AE medical records) will first be collected onto separate source documents before transcription or transferal into the data collection forms. The information in the data collection forms will then be entered directly into the electronic case report form (eCRF) in the study database.

Data collection forms derived from the eCRF will be provided and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

Sanaria and/or its designee will provide guidance to investigators on making corrections to the source documents and eCRF.

A copy of the cleaned and locked database will be provided to Sanaria and the Principal Investigator at the end of the study.

13.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the study site principal investigator or designee.

Data collection is the responsibility of the clinical trial staff at the study site under the supervision of the principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

StatPlus will serve as the Statistical and Data Coordinating Center for this study and, in collaboration with the study data manager, will be responsible for data management, quality review, analysis, and reporting of the study data.

13.2 Data Capture Methods

Clinical data (including AEs, concomitant medications, and reactogenicity data) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant Viedoc database managed by StatPlus. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

13.3 Types of Data

Safety assessments will be based on solicited reactogenicity reports collected from review of AE and SAE reports. Reactogenicity will be summarized by severity across the entire observation period

and into local and systemic symptoms. All AEs will be MedDRA® coded for Preferred Term and System Organ Class. The rate of AEs in aggregate, and by MedDRA® codes, will be computed for each vaccine group.

The number of SAEs is likely to be small in this study and will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (vaccination and AE), severity, and outcome for each event. The list will be by vaccine group. In the event that the number of all AEs is small, they will also be listed with the additional attribution of seriousness and with laboratory and clinical assessments.

13.4 Timing/Reports

Primary data analysis will occur after the primary study endpoint is reached 2 weeks following departure from natural exposure to malaria in eastern Indonesia, after which time study participants will continue to be followed for safety, immunogenicity and extended efficacy analysis. For the primary data analysis, study team members responsible for participant follow-up physical examinations and laboratory evaluations as well as study participants themselves will continue to remain blinded to study treatment assignment until the end of the study 24 weeks after return to home base.

Analyses of preliminary safety and immunogenicity data may occur after the 12-day follow-up visits have been completed following all three injections. These analyses will be further detailed in the study SOPs and/or statistical analysis plan.

Coding of AEs will be according to the MedDRA classification and will be managed by the statistical data and coordinating center as AE data is entered into the online database.

13.5 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of CDMRP. It is the responsibility of CDMRP to inform the investigator when these documents no longer need to be retained.

13.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), applicable regulations or SOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the study site, implemented promptly, and documented.

These practices are consistent with ICH E6 (R2):

- Section 4.5: Compliance with Protocol

- Section 5.0: Quality Management

- Section 5.1: Quality Assurance and Quality Control

- Section 5.20: Noncompliance

It is the responsibility of the study site to use continuous vigilance to identify and record deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All major deviations must be promptly reported to Sanaria.

Deviations from the protocol must be addressed in study participant source documents and reported in the study database. A completed copy of a protocol deviation form will be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations must be sent to the local IRB per their guidelines. The study site PI/study staff is responsible for knowing and adhering to their IRB requirements.

14 PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of Sanaria to register this trial in an acceptable registry. The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

15 LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AE	Adverse Event
AES	Advanced Encryption Standard
ALAT	Alanine Amino Transferase
ALU	Artemether / Lumefantrine
AMA-1	Apical Membrane Antigen 1
AS/AQ	Artesunate / Amodiaquine
ASAT	Aspartate Aminotransferase
BMI	Body Mass Index
BSPZV1	Bagamoyo Sporozoite Vaccine Trial #1
BSPZV2	Bagamoyo Sporozoite Vaccine Trial #2
CDC	United States Centers for Disease Control and Prevention
CDP	Clinical Development Plan
CDISC-ODM	Clinical Data Interchange Standards Consortium-Operational Data Model
CHMI	Controlled Human Malaria Infection
CFR	United States Code of Federal Regulations
CQ	Chloroquine phosphate
CRF	Case Report Form
CSP	Circumsporozoite protein
DVI	Direct Venous Inoculation
DHA-PP	Dihydroartemisinin piperaquine
EC-FMUI	Ethics Committee of the Faculty of Medicine, Universitas Indonesia
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EG	Equatorial Guinea
EGSPZV1	Equatorial Guinea Sporozoite Vaccine Trial #1
EGSPZV2	Equatorial Guinea Sporozoite Vaccine Trial #2
EGMVI	Equatorial Guinea Malaria Vaccine Initiative
EIMB	Eijkman Institute for Molecular Biology
ELISA	Enzyme-linked Immunosorbent Assay
ELISpot	Enzyme-linked Immunosorbent Spot
FDA	Food and Drug Administration (USA)
FMUI	Universitas Indonesia Faculty of Medicine
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSA	Human Serum Albumin
ICF	Informed Consent Form

ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICS	Intracellular Cytokine Staining
ID	Intradermal(ly)
ID	Identification
IDR	Indonesian rupiah (currency)
IFA	Immunofluorescence Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN	Interferon
IHI	Ifakara Health Institute (Bagamoyo, Tanzania)
IL	Interleukin
IM	Intramuscular(ly)
IMP	Investigational Medical Product
IND	Investigational New Drug
IOCRL	Indonesia and Oxford Clinical Research Laboratory
IP	Investigational Product
ISI	Inhibition of Sporozoite Invasion of Hepatocytes
IDES	Internet Data Entry System
ITN	Insecticide-treated Nets
IV	Intravenous(ly)
KSPZV1	Kenya Sporozoite Vaccine Trial #1
LLIN	Long-lasting Insecticide treated Net
LMIV	Laboratory of Malaria Immunology and Vaccinology
LNVP	Liquid Nitrogen Vapor Phase
MD	Medical Doctor
MDA	Mass Drug Administration
MeDRA	Medical Dictionary for Regulatory Activities
MM	Medical Monitor (= Research Monitor = Safety Monitor)
Mmed	Masters of Medicine
MOHSW	Ministry of Health and Social Welfare
MRI	Magnetic Resonance Imaging
MSc	Master of Science
MSP	Merozoite Surface Protein
MTA	Material Transfer Agreement
MVPs	Mass Vaccination Programs
NAI	Naturally Acquired Immunity
NF	Nijmegen <i>falciparum</i>
NIAID	National Institute of Allergy and Infectious Disease (NIH, Bethesda, USA)
NIH	National Institutes of Health (Bethesda, USA)
NS	Normal Saline Solution
OUCRU ID	Oxford University Clinical Research Unit Indonesia
OXTREC	Oxford Tropical Research Ethics Committee

PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
Pf	<i>Plasmodium falciparum</i>
PfCSP	<i>Plasmodium falciparum</i> Circumsporozoite Protein
PfLSA1	<i>Plasmodium falciparum</i> Liver Stage Antigen-1
PfMSP	<i>Plasmodium falciparum</i> merozoite surface protein
PfSPZ	<i>Plasmodium falciparum</i> Sporozoite(s)
PfSPZ-CVac	PfSPZ Chemoprophylaxis Vaccine (PfSPZ Challenge administered in conjunction with antimalarial chemoprophylaxis)
PfSPZ Challenge	live, fully infectious, aseptic, purified, cryopreserved <i>Plasmodium falciparum</i> sporozoites
PfSPZ Vaccine	live, radiation attenuated, aseptic, purified, cryopreserved <i>Plasmodium falciparum</i> sporozoites
PfSSP2/TRAP	<i>Plasmodium falciparum</i> sporozoite surface protein-2/thrombospondin-related adhesive protein
PhD	Doctor of Philosophy
PI	Principal Investigator
Pv	<i>Plasmodium vivax</i>
PRBME BRIN	Pusat Riset Biologi Molekuler Eijkman Badan Riset dan Inovasi Nasional = Eijkman Molecular Biology Research Centre - National Research and Innovation Agency
QA	Quality Assurance
QC	Quality Control
qPCR	Quantitative Polymerase Chain Reaction
RDT	Malaria Rapid Diagnostic Test
RM	Research Monitor (= Medical Monitor = Safety Monitor)
RTS,S	A recombinant protein subunit vaccine that includes part of the repeat region of the <i>Plasmodium falciparum</i> CSP (R), part of the region of CSP that includes thymus-derived lymphocyte epitopes (T), and the surface antigen of hepatitis B (S)
RUMC	Radboud University Medical Centre (Nijmegen, The Netherlands)
SAE	Serious Adverse Event
SC	Subcutaneous (ly)
SMC	Safety Monitoring Committee
SMO	Site Medical Officer
SOP	Standard Operation Procedure(s)
SPC	Summary of Product Characteristics
SPZ	Sporozoite(s)
SSL	Secure Sockets Layer
SUSAR	Suspected Unexpected Serious Adverse Reaction
SwissTPH	Swiss Tropical and Public Health Institute (Basel, Switzerland)
TB	Tuberculosis

TBS	Thick blood smear
TFDA	Tanzania Food and Drug Authority
TNF	Tumor Necrosis Factor
UMC	University Medical Center
USD	United States dollars (currency)
USMMVP	US Military Malaria Vaccine Program (Silver Spring, USA)
V	Day of immunization with PfSPZ Vaccine
VRC	Vaccine Research Center (NIAID, NIH, Bethesda, USA)
WHO	World Health Organization

16 PROTOCOL AMENDMENT HISTORY

1. Protocol versions 1.1 and 1.2 were for internal use at OUCRU.
2. Protocol version 1.3 was issued on 09 Feb 2018
3. Protocol version 2.0 was issued on 31 Jan 2019
4. Protocol version 2.1 was issued on 27 Sep 2019
5. Protocol version 2.2 was issued on 22 Nov 2021
6. Protocol version 2.3 was issued on 20 Jan 2022
7. Protocol version 3.0 was issued on 07 June 2022
8. Protocol version 3.1 was issued on 27 Sep 2022; see the summary of changes below:

Affected Section(s)	Summary of Revisions Made Red font = added language Strike-out font = removed language	Rationale
5.6 Exposure Follow-up	<p>OnAs soon as it is feasible after arrival, all participants will have a repeat thick and thin blood smear and qPCR sample collection (60 ul blood blotted onto filter paper and 500 ul into a microtube), blood blotted onto filter paper for genome sequencing (filter paper only, no 5 mL sample) and an 8-10 mL (target, 10 mL) blood sample for serology. Vital sign measurement, and as necessary, a focused physical examination, may be performed. If it is logistically preferable to obtain the serology sample at a separate time from the other blood samples, that is permitted.</p> <p>The thick and thin blood smears and qPCR samples will confirm that no study participant has acquired malaria at the time of departure from Sumatra or while traveling to Papua. The additional serology is required to establish a uniform pre-exposure baseline for all participants. Selecting a timepoint after arrival in Papua will adjust for any unanticipated differences between the PfSPZ Vaccine / placebo and PfSPZ-CVac / placebo groups in the time interval between the third</p>	<p>The additional blood sample for serology is required to establish a baseline antibody level for each study participant at the start of deployment. This will allow correlation of antibody levels just at the point where malaria exposure begins with protection during deployment. Obtaining a sample for baseline antibody was in the original protocol – a sample to be drawn 14 days after immunization. However, because most of the soldiers receiving PfSPZ Vaccine or placebo were deployed prior to V3+14 days, there was not enough time to obtain a post-vaccination / pre-deployment serology sample at V3+14, when the immune response from the third immunization would have matured. Instead, most samples were drawn at V3+3, V3+4, V3+6, V3+7, V3+8 and V3+9 days, before the antibody response to the third dose of vaccine could mature. However, we can still obtain this sample after the soldiers arrive in Papua, at which point all immune responses will have matured. Because the PfSPZ Vaccine and</p>

	<p>immunization and arrival in Papua. It will also more accurately reflect the maturation of the immune response after the third vaccine dose than samples taken earlier than fourteen days after the third dose prior to deployment, in case the deployment date does not allow a 14 day wait period before obtaining the serology sample. The serological data will be used to assess correlates of protection, an important study objective.</p> <p>These a Assessments will continue...”</p>	<p>PfSPZ-CVac groups should have the baseline serology measurement at the same time relative to the start of the exposure period, we need to draw this extra sample from all participants on arrival in Papua – both V and CV groups.</p> <p>How does this affect the risks associated with the study? Including the extra 10 mL, the total amount of blood drawn for the PfSPZ Vaccine / placebo group will be 248 mL, which is less than the 264 mL the participants agreed to at the start of the study as specified in the consent form. For the PfSPZ-CVac / placebo group, the total amount of blood drawn will be 274 mL, which is 10 mL more than the 264 mL the participants agreed to at the start of the study, indicating a very slight increase in risk.</p>
6.3.1.5 Malaria Smears	PfSPZ Vaccine: At time of screening, at time of first immunization, 2 weeks after the last immunization, on arrival to the field site and every 4 weeks during Exposure....	Clarify when the malaria samples should be taken per protocol.
6.3.1.5 Malaria Smears	PfSPZ-CVac: At time of screening, at time of each immunization, +7, +8, +9, and +12 days after the first and second immunizations, +7, +8, +9, and +14 days after the third immunization, on arrival to the field site and every 4 weeks during Exposure.....	Same as above.
6.3.1.6 qPCR	Adding 2. Exclusion of parasitemia at the beginning of the Exposure period.	In the original protocol design all study participants were to undergo blood sampling within the first 28 days of arrival in Papua to begin evaluations for malaria. In order to have all participants begin the exposure period of the trial on an equal basis – free of malaria – it is

		important to test for malaria using a very sensitive blood test at the beginning of the exposure period.
6.3.1.6 qPCR	PfSPZ Vaccine: At time of screening, at time of first immunization, 2 weeks after the last immunization, on arrival to the field site and every 4 weeks during Exposure...	Same as above.
6.3.1.6 qPCR	PfSPZ-CVac : At time of screening, at time of each immunization, +7, +8, +9, and +12 days after the first and second immunizations, +7, +8, +9, and +14 days after the third immunization, on arrival to the field site and every 4 weeks during Exposure...	Same as above.
6.3.2.1 Serology assays	These studies will be done on samples collected at baseline (pre-immunization), +14 days after the third immunization, at the beginning and end of the Exposure period and at the beginning and end of the Post-Exposure period.	Clarify when the serology samples should be taken per protocol.

9. Protocol version 4.0 was issued on 22 May 2023; see the summary of changes below:

Affected Section(s)	Summary of Revisions Made Red font = added language Strike-out font = removed language	Rationale
Global change throughout document, wherever "EOCRU" was mentioned.	EOCRU changed to OUCRU ID	Administrative change consistent with new status of the Oxford Clinical Research Unit Indonesia which is now affiliated with Faculty of Medicine University of Indonesia.
Global change throughout document, wherever "EIMB" was mentioned.	EIMB changed to PRBME-BRIN	The name of EIMB is changed from Eijkman Institute of Molecular Biology to Eijkman Molecular Biology Research Centre – National Research and Innovation Agency
Protocol Summary; Sections 2.1 and 2.2.	Malaria cases identified in soldiers participating in the Yahukimo Task Force and diagnosed by rapid diagnostic	The original protocol did not anticipate that the soldiers stationed in Papua might be sent away from their posts on special missions where, for

	<p>test (RTD) or by thick blood smear (TBS) performed by regional health care facilities <u>will be counted as valid endpoints</u>. Consequently, the protocol language is amended to allow this, as (previously) endpoints required microscopy performed by OUCRU ID's certified microscopists. The following changes were made to the protocol:</p> <p>(1) Primary Objective #2 changed: "To assess the protective efficacy (vaccine efficacy = VE) of PfSPZ Vaccine and PfSPZ-CVac against first clinical malaria cases caused by <i>P. falciparum</i> (Pf) identified by thick blood smear (TBS) microscopy or rapid diagnostic testing (RDT) in naturally exposed Indonesian soldiers." Similar changes were made to secondary objectives 1, 2, 3 and exploratory objective 1.</p>	<p>security reasons, the OUCRU ID team would not be allowed to accompany them. However, this did happen: 38 soldiers were sent to Yahukimo District as part of a special Task Force, accompanied by Army paramedical personnel but no OUCRU ID clinical team members. The paramedics were trained on the conduct of Rapid Diagnostic Tests (RDTs), which were subsequently used to identify many malaria infections. We would like to be able to count these as valid endpoints contributing to the primary efficacy objectives of the protocol.</p>
Protocol Summary; Section 2.2, and 2.3.3.	<p>A new exploratory objective was added (now exploratory objective #2): "To assess the VE of PfSPZ Vaccine and PfSPZ-CVac for reducing the number of asymptomatic Pf and Pv malaria infections."</p>	<p>WHO has advised that endpoints for malaria vaccine trials should include not just first or only infections, but all infections. Therefore, this exploratory objective has now been added.</p>
Section 2.3.2	<p>More details are added regarding how adverse events will be tallied, including adding language that the number of participants experiencing an AE after each dose will be tallied, not just the number of participants experiencing AEs after any of the three doses. Another detail added is that the</p>	<p>AEs will be tallied after each dose to see if there are trends of increasing or decreasing AE frequency with repeated dosing. AE's in the PfSPZ-CVac/placebo group will be broken into three periods for the following reasons: days 1-6 after each immunization are when AEs can be caused by sporozoites and liver stages; days 7-10 after immunization</p>

	<p>AEs experienced by participants on the PfSPZ-CVac/placebo group will be divided into those AEs occurring during days 1-6 after each vaccination, those AEs occurring days 7-10 after each vaccination, and those AEs occurring at other times during the AE collection periods.</p>	<p>are when AE's can be caused by transient parasitemia; and the other time periods are when AE's can be caused only by chloroquine. By looking at the time periods separately, we may learn more about the individual reactivity of each parasite stage.</p>
Section 2.3.2	<p>The official endpoint of the primary surveillance period is changed from 10 days after leaving Jayapura to the last day before departure that the OUCRU ID team has access to the participants.</p>	<p>The OUCRU ID team will not be accompanying the participants on the ship; consequently, it will be difficult to officially diagnose malaria on the ship as an endpoint, although the paramedics will have RDTs and will be able to make a diagnosis and institute treatment.</p>
Section 3.1	<p>The timing of the primary analysis is changed from after the full completion of the study to after completion of the primary deployment. Previously, the analysis after completion of the primary deployment was termed an "interim analysis" and the analysis at the end of the study was termed the "final analysis." Now the former is primary, and the second is an "additional analysis." To do this, the wording was changed as follows:</p> <p>"16). The first unblinded analysis will be conducted for safety endpoints and efficacy against first infection with Pf and Pv shortly after the battalion returns to home base. An interim Study ReportThe primary analysis will be prepared at this time along with a Study Report. The research team conducting post-exposure surveillance will remain</p>	<p>This change has been made to speed up the availability of the primary vaccine efficacy analyses, as the team is interested in publishing our results promptly, particularly if there is significant vaccine efficacy.</p>

	<p>blinded as to vaccine or placebo assignment of the participants.</p> <p>17). The finalAdditional analyses will be conducted after the participants have completed the 24-week follow up after returning from Eastern Indonesia. A second interim and used to update the Study Report will be prepared updating the safety finding and reporting with the results of the Pv relapse component and with the additional analyses performed, such as those performed after confirmation of endpoints. This will constitute the final Clinical Study Report will then be prepared.</p>	
Section 3.3, 5.3	<p>The following words have been added with respect to chloroquine administration: “In case of delays in immunizations, CQ administration will continue with additional weekly doses so that the last dose is no less than +5 days after the third injection.” Also: “As stated above, dose may be adjusted slightly depending on the mg content of the tablets and to account for delays resulting from training periods away from the home base.”</p>	This change fills in missing details from the original protocol.
Table 8	<p>The following footnote is added to the table: “* It is possible that soldiers will be sent on missions to remote areas during deployment, and that members of the OUCRU ID team will not be allowed to accompany the soldiers for security and safety reasons. If this circumstance arrives, the OUCRU ID will train</p>	This adjustment is needed to account for the Yahukimo Task Force mission.

	the Army medics accompanying the soldiers on the mission to collect as many of the above samples as possible.”	
Section 5.11 and 6.3.1.5.	The following change was made: “Blood smears from asymptomatic individuals will be read retrospectively later in the deployment or after deployment is finished.”	The reason for adding “later in deployment” is that, in fact, the team HAS started to read the TBS from asymptomatic soldiers while the deployment is still underway. They are being read months after they were collected, so there is no danger of influencing the clinical decisions of the clinical team. In addition all results are being kept from the clinical team, so as not to introduce any bias into their evaluations of the participants. Starting to read these smears now will mean that reading will be completed sooner.
Section 6.3.1.1	The following words are added: “During deployment, hospital-based or commercial laboratories will be used if needed for laboratory testing of individual soldiers with clinical concerns.”	This corrects an omission from the original protocol.
Table 15	Minor changes have been made to the toxicity grading for abnormal laboratory values. With these changes instituted, the toxicity grading can now be referenced as “*Grading scales derived from ‘Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials’, US FDA, 2007, and adjusted to locally derived normal ranges.” This reference has been added as a footnote to Table 15.	This change has been made because no one on the OUCRU ID or Sanaria teams could provide a clear reference for the toxicity grading in the protocol, which were put together by OUCRU ID staff members who left OUCRU ID several years ago.

<p>Section 9.4.1</p>	<p>The following wording has been added: <u>“Added with Protocol Version 4.0. Due to the fact that all PfSPZ Vaccine / placebo immunizations were performed out of window (significantly delayed), a “modified per protocol” (mPP) cohort is defined that while requiring first and second immunizations to be within window, the third immunizaion may be delayed.”</u></p> <p>And</p> <p><u>“Added with Protocol Version 4.0. Since the original protocol was written in 2017, Sanaria has started to perform primary analyses on a “modified intention to treat (mITT) population defined as having received all three vaccinations, even if not necessarily within window or even if the full volume of injectate was not administered. The use of mITT for the primary analysis applies to Phase 1 and Phase 2 trials. So that the results of the IDSPZV1 trial will be comparable to other trials, the same convention is adopted here. Consequently, the mITT cohort will be the primary analysis cohort.”</u></p>	<p>This first change is necessitated by the fact that no PfSPZ Vaccine / placebo recipients were administered all three immunizations within allowed time windows (due to the particle issue). The third immunization was delayed in all of them.</p> <p>The second change will increase the comparability of the study results with other studies sponsored by Sanaria.</p>
<p>The following changes are made due to the limited resources available to complete the post-redeployment protocol procedures; they are designed to save costs without compromising safety. Cost-cutting is part of the rationale for each of the following changes. The rationale column includes an explanation regarding how the change will or will not affect safety or scientific objectives. Note that ALL wording for these changes is written in the form of options, allowing the protocol to be executed as originally written (if supplemental funding is identified), or executed as amended (if supplemental funding is not identified).</p>		

Protocol Summary	<p>Changes to surveillance procedures during the post re-deployment surveillance period:</p> <p>(1) No automatic thick blood smears (TBS) in asymptomatic soldiers; (2) Directly observed treatment is not required for <u>every</u> dose of antimalarial medication; (3) Follow-up post treatment with DHA-PP on days +7, +14, +21 and +28 is not required if participant is clinically well. As a general description of this (with more detail provided in other sections as described below), the following changes were made to the <u>protocol summary</u>: "Follow-up during period #2 will be the same with respect to passive case detection but may or may not include active surveillance. During follow-up period #2, unlike follow-up period #1, with the addition of there will be supervised radical cure with primaquine for participants having TBS confirming confirmed infection by Pv."</p>	<p>(1) The automatic TBS performed every four weeks during the <u>primary</u> surveillance period <u>in Papua</u> was not a safety feature. Rather, it was designed to identify asymptomatic infections, most of which likely presented soon thereafter as symptomatic infections, and were diagnosed and treated. We will know if any such asymptomatic infections remained asymptomatic and therefore can be counted as new, asymptomatic infections, once the TBS from these asymptomatic soldiers are read. Our expectation, based on the malaria-naïve status of the soldiers, is that all such asymptomatic infections became symptomatic soon thereafter. The contribution to the study of this effort to identify new, persistently asymptomatic infections was therefore likely minimal. Since we expect that all Pv relapses presenting post-redeployment will similarly present as symptomatic infections, and since counting asymptomatic relapses is not a protocol objective (see objectives), there is no need to obtain TBS from asymptomatic soldiers post redeployment. (2) According to general medical practice including military medical doctrine, all doses of medication taken by healthy individuals do not require observation. Rather, the soldiers are given written instructions, and expected to take the medication as prescribed on their own cognizance. This change therefore is consistent with routine medical practice. (3) Similarly, under routine medical practice, when an individual is treated for an infection, return visits</p>
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		to the physician generally are not required as long as the individual recovers, remains well, and takes the prescribed medication as directed. Therefore, we believe that these changes can be made without compromising safety.
Sections 2.3.2, 2.3.3, 6.3.1.6.	(1) The requirement that all endpoints be confirmed by the FDA-approved qPCR assay (Sean Murphy, University of Washington) is removed; (2) An analysis of vaccine efficacy where endpoints ARE confirmed is re-instituted as an exploratory objective; (3) A provision is added that endpoints diagnosed by RDT can be confirmed by either microscopy or PCR.	Due to resource limitations, it may not be possible to confirm endpoints using the FDA-approved qPCR assay (Sean Murphy, University of Washington). We also would like it to be possible to confirm the RDT results used in Yahukimo with a PCR assay.
Section 5.2	The following paragraph was added: "Addendum added with Version 4.0 of the protocol: A shortage of funds for completing the trial as planned may result in the inability to collect most of the research samples slated for the post-redeployment period. In addition, the final set of safety laboratory testing may be omitted, as the scheduling of these laboratory tests six months after redeployment and more than a year from vaccination was primarily for research, not safety purposes. However, the first set of safety laboratory testing post-redeployment will be conducted (with an option to conduct the tests in Papua prior to redeploment rather than after returning to the home base in Bangkinang), as this is important	This explains how resource limitations are affecting the protocol and why omitting the last set of chemistry and hematology tests (those scheduled for the end of the post-redeployment surveillance period) does <i>not</i> constitute a safety risk for the research participants. The chemistry and hematology test scheduled for the beginning of the post-redeployment surveillance period ARE important from a safety perspective and are being retained. However, the option is being provided to conduct these tests in Papua at the end of the deployment period, rather than after returning to Bangkinang.

	from the perspective of participant safety. For example, in a prior study of a battalion redeploying back to its home base in Malang after deployment to Papua, 12% of soldiers had grade 1 or grade 2 anemia at the time of their return, likely as a result of exposure to malaria infections during the deployment.”	
Section 5.4 (DHA-PP)	The word “approximately” is added to the following: “DHA-PP will be the first line drug for treatment of Pf, Pv or other species of malaria. It is administered over three consecutive days for a total of three doses taken at approximately the same time each day.”	This was an omission in the original protocol. The drug combination does not need to be administered at precisely the same time each day.
Table 7	The rows for “physical examination,” “physical assessments,” and “adverse event data collection” have had the following words added: “during post-exposure period, only if ill” meaning that it is not necessary to examine soldiers or formally record adverse events if the soldiers are healthy during the post redeployment surveillance period.	Collecting data on healthy soldiers who are not experiencing any signs or symptoms is not needed during the post redeployment surveillance period, and omitting this requirement saves resources.
Table 7. For the change in cell mediated immunity samples, see also Section 6.3.2.2.	The following blood sample collections have been made optional: (1) biochemistry and hematology testing at the end of the study (as described two rows above); (2) automatic parasitology samples every 4 weeks in asymptomatic soldiers; (3) A serology sample at the end of the study; (4) PMBC samples	As explained two rows above, the biochemistry and hematology sampling at the end of the study are not needed for safety reasons, as that time point is six months after redeployment and more than a year after immunization. Likewise, the parasitology sampling in asymptomatic participants is not necessary in order to assure their

	at the beginning of the post redeployment surveillance period and at the end of the study.	safety (for example, during the primary deployment period, although these samples were collected, they were not analyzed in real time). The serology samples to be collected at the beginning of the post redeployment surveillance period WILL be collected, but it will be done in Papua (see next entry). The serology samples to be collected at the end of the study, AND the PBMC samples to be collected at the beginning of the post redeployment surveillance period and at the end of the study are research assays and are not needed for safety. Not performing them will save resources.
Table 7	An option is added allowing the post redeployment serology samples to be collected in Papua prior to the soldiers boarding the ship.	This saves resources because the team for drawing these samples is in place in Papua but will not be in place in Sumatra. From a scientific perspective, it does not matter if the samples are drawn before the soldiers board the ship, or after they arrive back at home base in Bangkinang.
Table 8	The following footnote is added to Table 8: “** If D7 TBS is negative and participant did not have symptoms, TBS from D14, D21, D28 do not need to be read unless symptomatic.”	These visits are not needed from a safety perspective and changing their status from required to optional saves resources. All soldiers will be strictly instructed to report any signs or symptoms developing during the post treatment follow-up period, and they will be seen immediately if this occurs.
Section 5.7	Section 5.7 is the main section on Post Exposure Follow-up (= post redeployment surveillance). The section has been edited to reflect the new plan as described above. Specifically, the following changes have been made: “The primary means of follow-up will be passive case detection. In other words, soldiers will be	As described above, the resource-sparing plan for the post redeployment surveillance period focuses on the identification and immediate evaluation of <u>all sick soldiers</u> . What has been changed to optional is the evaluation of asymptomatic soldiers who have no signs or symptoms of illness.

	<p>instructed to report any illness to the OUCRU ID clinical team, and if such illness is reported, the OUCRU ID team will interview the soldier, obtaining vital signs, a medical history, a physical examination if indicated, and finger-prick blood for a TBS. If the TBS is positive, the soldier will be treated with DHA-PP per national guidelines, including administration of primaquine (0.5 mg/kg/day for 14 days) in the case of <i>P. vivax</i>, to prevent any further <i>P. vivax</i> relapses. Administration of these medications will not be supervised, and follow-up TBS will not be done unless there is persistence of clinical symptoms.</p> <p>In addition to passive case detection, there potentially will may be active case detection. This will be done by conducting visits to every soldier every 4 weeks (instead of every two weeks as was done during deployment), if staffing and resources are sufficient to conduct these visits. Each visit will include at a minimum active questioning regarding malaria symptoms and fever. If resources allow, there may be additionally the acquisition of a blood sample for blood smear, and if possible, blood blot on filter paper for qPCR, blood in a microtube for qPCR, blood blot on filter paper for genome sequencing. In addition vital sign measurement may be done, and it will be determined if the participant has</p>	
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	<p>had symptoms consistent with malaria during the past 4 weeks and if they received treatment for malaria. If determined necessary, focused physical exam may be done at the discretion of the study clinician. If the blood smear is positive and personnel and facilities are available, follow up will be as above for Exposure Follow-up (section 5.6), including the 5 mL sample for in-depth genome sequencing, but this is now optional given the resource restrictions imposed on the study.</p> <p>Follow-up of soldiers found to have malaria will include at minimum provision of DHA-PP for a three day course of therapy and of primaquine for a 14 day course of therapy. The soldier may take these medications on his own cognizance. The additional visits and sampling described in Table 8 are not required but will be followed if resources are available."</p>	
Section 6.1.3	<p>This section also describes post redeployment follow-up and similar changes are made, such as adding the words "Passive case detection will be used through out the post-redeployment follow-up period. If resources allow, there will additionally be Routine 4-weekly visits and examinations."</p> <p>With respect to the hematology and chemistry tests originally scheduled for the end of the</p>	It is important that this rationale be provided in the amended protocol.

	study, it is written: “The CBC and chemistry tests at this visit are primarily motivated by scientific, not safety reasons. Specifically, after six months back at home base, there are no residual safety concerns related to either immunization, which took place more than a year before, or to deployment in Papua, which ended six months before.”	
Section 5.8	The final study visit is made optional by the following changes in wording: “The final study visit will occur approximately 24 weeks after return for normal duty at their home base, if resources allow. However, the final study visit is not required for safety reasons because soldiers will already have been surveilled for 24 weeks after their redeployment to their home base, enough time to identify relapsing <i>P. vivax</i> infections. If the final study visit does take place, evaluations to be done during the final study visit are listed in the Study Procedures/Evaluations section (see Table 7). If possible, these will include the full parasitology assessment (including 5 mL for in-depth genome sequencing) but all blood collection and testing is optional, depending on staffing and resources available.	Conducting the last study visit is not required for either safety or scientific reasons. What is important during the post redeployment surveillance period is detecting and treating all Pv relapses, and indeed this will be done.
Section 9.3.7	Analysis of strain-specific efficacy and selection has been specified as “optional.”	Not performing this exploratory objective saves resources. It has no impact on safety.
Section 12.5	The following wording is added: “Compensation may or may not	This change conserves resources. Note that in the resource constrained

	be offered during the six month post-redeployment surveillance period, depending on resources available.”	setting, blood will ONLY be drawn from study participants during the post redeployment surveillance period when soldiers are sick (other than the initial hematology and chemistry safety laboratory tests). Since this blood drawing would normally be done (e.g., for the diagnosis of relapsing Pv malaria) even if the soldiers were not in the study, the blood drawing is not considered different from normal clinical care. Therefore, compensation is not felt to be required. However, compensation WILL be done for the chemistry and hematology safety labs drawn at the beginning of the post redeployment observation period, per routine practice.
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18 APPENDICES

18.1 Appendix A

Gaziano Scale for Cardiovascular Risk

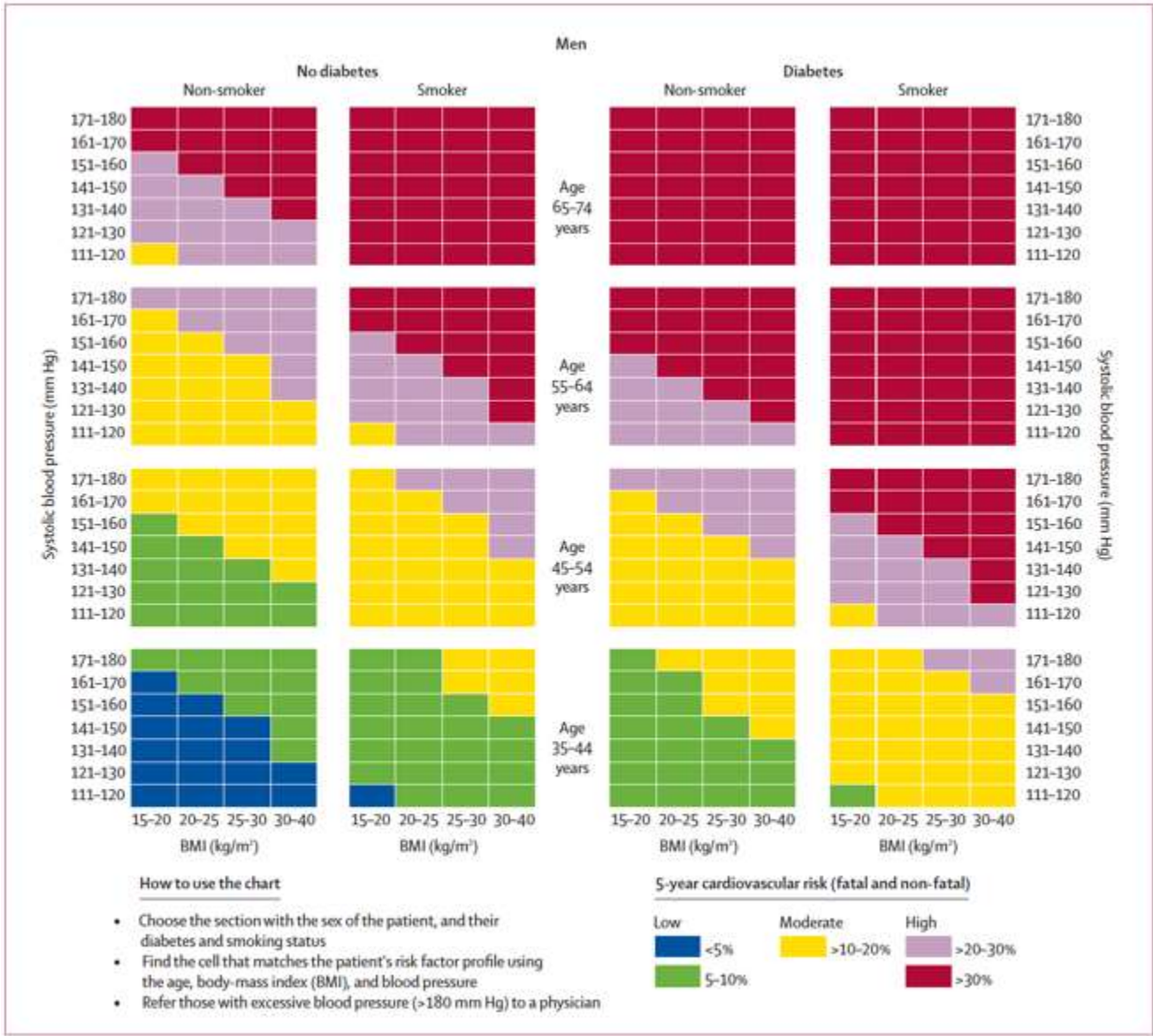


Figure 5: Risk prediction chart for cardiovascular disease using non-laboratory-based measures (men)

Laboratory-based versus non-laboratory-based method for assessment of cardiovascular disease risk: the NHANES I Follow-up Study cohort

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