Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Immunogenicity, and Protective Efficacy of Two Regimens of Radiation Attenuated *Plasmodium falciparum* NF54 Sporozoites (PfSPZ Vaccine) During Natural Transmission Season in Healthy African Adults in Mali

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Laboratory of Malaria Immunology and Vaccinology (LMIV)
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IND Sponsor:
Sanaria, Inc.
IND: BB-IND # 14826

Principal Investigators:
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LIST OF ABBREVIATIONS

AGC	absolute granulocyte count
AE	adverse event/adverse experience
AL	artemether/lumefantrine
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ASAQ	artesunate-amodiaquine
BS	blood smear
CBC	complete blood count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CHMI	controlled human malaria infection
CoA	Certificate of Analysis
Cr	creatinine
CSP	circumsporozoite protein
DSMB	Data Safety Monitoring Board
DVI	direct venous inoculation
EC	Ethics Committee
ECG	electrocardiogram
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FMPOS	Faculty of Medicine, Pharmacy and Odonto-Stomatology, Bamako, Mali
GCP	good clinical practice
GMP	good manufacturing practice
Hb	hemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HRPP	Human Research Protection Program
HSA	human serum albumin
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IFA	immunofluorescence assay
IND	Investigational New Drug
IPTp	intermittent preventive treatment during pregnancy
IRB	Institutional Review Board
ISM	Independent Safety Monitor

ITN	insecticide-treated bed net
ITT	intention to treat
IV	intravenous
LMIV	Laboratory of Malaria Immunology and Vaccinology
LNVP	liquid nitrogen vapor phase
MRTC	Malaria Research and Training Center
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOCI	New Onset of Chronic Illness
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PBS	phosphate buffered saline
Pf	*Plasmodium falciparum*
PfCSP	*Plasmodium falciparum* circumsporozoite protein
PfSPZ	*Plasmodium falciparum* sporozoite
PfSPZ Challenge	viable *Plasmodium falciparum* sporozoites produced by Sanaria, Inc.
PfSPZ Vaccine	radiation attenuated *Plasmodium falciparum* Sporozoites produced by Sanaria, Inc.
PI	Principal Investigator
qPCR	quantitative polymerase chain reaction
QTc	QT interval
SAE	serious adverse event
SAR	suspected adverse reaction
SNP	single-nucleotide polymorphism
SOP	standard operating procedure
SUSAR	Serious and Unexpected Suspected Adverse Reaction
UP	unanticipated problem
UPnonAE	unanticipated problem that is not an adverse event
USD	US dollar ($)
VE	vaccine efficacy
VRC	Vaccine Research Center
WBC	white blood cells
WHO	World Health Organization

PROTOCOL SUMMARY

Full Title:	Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Immunogenicity, and Protective Efficacy of Two Regimens of Radiation Attenuated *Plasmodium falciparum* NF54 Sporozoites (PfSPZ Vaccine) During Natural Transmission Season in Healthy African Adults in Mali
Short Title:	Two regimens of PfSPZ Vaccine in Mali, Africa
Clinical Phase:	Phase 1
IND Sponsor:	Sanaria, Inc.
Clinical Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Conducted by:	Malaria Research and Training Center (MRTC), in collaboration with the Laboratory of Malaria Immunology and Vaccinology (LMIV)
Principal Investigators:	Halimatou Diawara (MRTC) Patrick Duffy (National Institute of Allergy and Infectious Diseases [NIAID] / National Institutes of Health [NIH])
Vaccine Sample Size:	N=210 (Main study)
Accrual Ceiling:	N=700 Reconsent for Booster phase: Previously enrolled and vaccinated N=200
Study Population:	Healthy adults (18 to 35 years of age), residents in Ouelessebougou and surrounding villages, Mali
Accrual Period:	Approximately April 2018 to April 2019 (MAIN PHASE) Approximately April 2019 to March 2020 (BOOSTER PHASE)
Study Design:	A randomized double blind, placebo-controlled study to assess the safety, tolerability, immunogenicity and protective efficacy of 9 x10^5 irradiated *Plasmodium falciparum* sporozoite (PfSPZ Vaccine) in healthy adults when given at 0, 8 and 16 weeks and at 0, 1 and 4 weeks. Participants will be randomized into four arms. At the completion of the follow up period in the first transmission season, the study will remain blinded and will assess the safety, tolerability, immunogenicity and protective efficacy of a 4th vaccination (booster dose). *Arms 1* and *2* will receive 9 x10^5 PfSPZ Vaccine whereas *Arm 3a* and *3b* will receive normal saline. The booster phase will evaluate efficacy in the subsequent transmission season.

Arm 1: (n=70) will receive 3 doses of PfSPZ Vaccine (9×10^5) via direct venous inoculation (DVI) at 0, 8, and 16 weeks. A 4th dose at 38 weeks post 3rd vaccination

Arm 2: (n=70) will receive 3 doses of PfSPZ Vaccine (9×10^5) via DVI at 0, 1 and 4 weeks. A 4th dose at 38 weeks post 3rd vaccination

Arm 3a: (n=35): Will be the control for Arm 1. Volunteers will receive 3 doses of placebo saline injection via DVI at 0, 8 and 16 weeks. A 4th dose at 38 weeks post 3rd injection

Arm 3b: (n=35): Will be the control for Arm 2. Volunteers will receive 3 doses of placebo saline injection via DVI at 0, 1 and 4 weeks. A 4th dose at 38 weeks post 3rd injection

- All injections will be administered by DVI.
- All volunteers will receive antimalarial treatment with artemether/lumefantrine (AL) 2 weeks prior to 3rd injection and 4th injection
- Participants will be monitored for safety, immunogenicity, malaria infection and disease during the follow-up period.

Study Duration:

MAIN PHASE

Start Date: Approximately April 2018

End Date: Approximately April2019

BOOSTER PHASE

Start Date: Approximately April 2019

End Date: Approximately March 2020

Study Agent Description: Radiation attenuated, aseptic, purified, vialed, cryopreserved, NF54 *Plasmodium falciparum* sporozoites (referred to as PfSPZ Vaccine) produced by Sanaria, Inc. will be administered via DVI at the doses and time intervals specified above.

Primary Objective:

Safety:

1. Assess safety and tolerability of PfSPZ Vaccine primary series in healthy Malian adults when given at 0, 8 and 16 weeks and at 0, 1 and 4 weeks (Main phase)
2. Assess safety and tolerability of PfSPZ Vaccine booster dose (4th dose) in healthy Malian adults (Booster phase)

Secondary Objectives: **Protective Efficacy:**

1. Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian adults when given at 0, 8 and 16 weeks and at 0, 1, and 4 weeks.
2. Assess the efficacy of PfSPZ Vaccine booster dose (4th dose) in healthy Malian adults

Exploratory Objectives: **Immunogenicity**

- Characterize and compare host immune responses to malarial antigens, and host proteomic profiles and transcriptomes in African adults prior to and after vaccination with PfSPZ Vaccine and between uninfected and infected subjects.
- Explore the impact of PfSPZ Vaccine on the genotype and transcriptome profile of parasites isolated from study subjects.

Primary Endpoints: **Safety:**

1. Incidence of local and systemic adverse events (AEs) graded by severity occurring within 7 days after each vaccine administration (Main phase).
2. Incidence of local and systemic adverse events (AEs) graded by severity occurring within 7 days after vaccine administration (Booster phase).

Secondary Endpoints: **Protective Efficacy:**

1. *P. falciparum* blood stage infection defined as time to first positive blood smear (detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL) starting immediately following 3rd injection (Main Phase).
2. *P. falciparum* blood stage infection defined as time to first positive blood smear (detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL) starting immediately following 4th injection (Booster Phase).

Exploratory Endpoints: **Immunogenicity**

- Measurement and comparison of various immunological parameters:
  - in the same subject prior to and after vaccination
  - between parasitemic and aparasitemic subjects
- Determination of the genotype of parasites isolated from study subjects, using microsatellite typing, sequencing, and/or single-nucleotide polymorphism (SNP) chip assays.

PRECIS

It is known that humans can be protected against malaria by repeated immunization with radiation-attenuated sporozoites. Sanaria, Inc. has developed a process for manufacturing, in compliance with current Good Manufacturing Practices (cGMPs) aseptic, purified, radiation-attenuated cryopreserved sporozoites from a well-characterized isolate of *Plasmodium falciparum* (Pf) (Hoffman et al., 2010). This product, which is called PfSPZ Vaccine, can be administered by needle and syringe.

A collaboration among the Malaria Research and Training Center (MRTC, Mali), the Laboratory of Malaria Immunology and Vaccinology (LMIV) National Institute of Allergy and Infectious Diseases (NIAID), and Sanaria, Inc. (Sissoko et al., 2017) has shown that sterile protection against naturally occurring malaria infection can be achieved. In this study, five doses of 2.7×10^5 PfSPZ during the dry season resulted in protective efficacies of about 48% by time to first positive blood smears (BS) and about 29% by proportion of participants with at least one positive BS during a full malaria transmission season (20 weeks), higher than those reported for other malaria vaccine candidates (Sissoko et al., 2017).

A follow up study in 2015 (ClinicalTrials.gov Identifier: NCT02627456) that reduced the number of vaccinations (from 5 to 3) while increasing the dose of sporozoites at each vaccination (2.7×10^5 to 1.8×10^6 PfSPZ Vaccine) was conducted. Preliminary results show that 42 of 55 (77.8%) participants from the placebo group and 32 of 54 participants (58.1%) from the vaccine group developed *Pf* infection. Per protocol, the vaccine efficacy (VE) was 51% (p=0.004, 95% CI 20-70) by time-to-infection analysis (intention to treat [ITT] 39%, p=0.033) and 24% (p=0.031, 95% CI 2-41) by proportional analysis (ITT 22%, p= 0.041), similar to the previous study.

Studies are ongoing to establish a vaccination regimen, optimum dose and schedule, that will lead to improved sterile protection in endemic regions. Preliminary results from more recent studies in malaria-naïve and malaria-experienced participants have shown that 9.0×10^5 PfSPZ Vaccine dose per vaccination (lower than 1.8×10^6 used in studies above) in a three-dose regimen may be an optimal dose for immunization. In addition, there is emerging evidence that a condensed, more practical regimen may also lead to development of sterile immunity. This proposed study is therefore designed to assess safety, immunogenicity and protective efficacy of two separate three-dose vaccination regimens during natural transmission season.

Participants in the main phase were randomized into arms receiving either PfSPZ Vaccine or normal saline injections. Each group received three doses of the respective injection. After completion of the follow up in the main phase, all participants that are still enrolled in the study will be offered continued participation to receive a booster dose of the vaccine (4^{th} dose) with 9.0×10^5 PfSPZ or normal saline (depending on the group they were originally randomized to) at approximately 10 months post 3^{rd} vaccination. The booster dose is timed prior to ensuing malaria transmission season. Participants will be followed, similarly to the follow up during the main phase, for safety and vaccine efficacy for approximately 6 months during this second transmission season.

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 The Need for a Malaria Vaccine

In recent years, the fight against malaria has received a considerable boost, with major funding organizations proposing eradication (Grabowsky, 2008; Roberts & Enserink, 2007). The increased use of several proven cost-effective measures available to reduce malaria such as insecticide-treated bed nets (ITNs), indoor residual spraying (IRS), artemisinin-based combination therapy (ACT) and intermittent preventative treatment during pregnancy (IPTp) over the last decade is thought to have contributed to the recently observed decline in clinical malaria in many parts of Sub-Saharan Africa (Ceesay et al., 2008; O'Meara, Bejon, et al., 2008; O'Meara, Mwangi, et al., 2008; B. Walther & Walther, 2007). However, despite concerted efforts by the World Health Organization (WHO), the United Nations Children's Fund, and the World Bank's Roll Back Malaria initiative to scale up these measures, the WHO Malaria Report 2017 estimates that only 19% of pregnant women received the recommended three doses or more of IPTp, and although the ownership of ITNs has increased to 80% in 2016 compared to 50% in 2010, the proportion of households with an adequate number of ITNs remains at 46%. Additionally, WHO estimates that 6 billion US dollars (USD) per annum would be necessary to sustain and increase current control efforts; however, in 2016, only 2.7 billion USD were available (Das & Horton, 2010; WHO, 2017; *World malaria report 2011*, 2011). It is also worth noting that mortality attributable to malaria remained stable in West Africa, and even increased in East and South Africa throughout the last 2 decades (Korenromp, Williams, Gouws, Dye, & Snow, 2003; Ndugwa et al., 2008). Moreover, recent estimates suggest that malaria mortality in individuals aged five years or older has been grossly underestimated (Murray et al., 2012). Collectively, these data raise concern for the hope of eradicating malaria with currently available means and highlight the urgent need to develop an efficient vaccine to eliminate malaria. Vaccination targeted toward the clinically silent liver-stage of infection would ideally provide sterile protective immunity, preventing progression to blood-stage infection and clinical disease, and transmission of parasites to mosquitoes. This is the target of leading vaccine strategies including the partially effective recombinant circumsporozoite protein (CSP)-based Mosquirix (RTS,S) vaccine, currently in implementation studies throughout Africa. Subunit vaccines of this type utilize conserved antigenic targets to elicit protection against sporozoite migration, hepatocyte infection and intrahepatocytic parasite replication. Mosquirix has protected malaria-naïve adults against controlled human malaria infection (CHMI) with *Plasmodium falciparum (Pf)* and reduced malaria-associated episodes in children living in malaria endemic areas, but the level and length of immunity seen is relatively modest (Agnandji et al., 2011; Rts, 2015; Stoute et al., 1998). Although follow-up studies of Mosquirix (RTS,S) vaccine have shown decreases in clinical malaria in vaccinated children, at 6 months post-vaccination follow-up, the levels of antibodies were not significantly different between vaccinated and unvaccinated children and did not predict protection against clinical malaria in the following 12 months (Campo et al., 2014). The mechanism by which Mosquirix (RTS,S) and other sporozoites (SPZ) and liver-stage vaccine strategies confer protective immunity is still under investigation.

1.2 Challenges of Developing a Malaria Vaccine

Designing a malaria vaccine is not straightforward (M. Walther, 2006). In contrast to most infectious diseases, natural sterile immunity to *P. falciparum* has not been demonstrated. Nature

does not provide a clear template for protective immune mechanisms that a vaccine could mimic. The inability to completely clear parasitemia or to prevent re-infection may be the result of an array of sophisticated immune evasion strategies used by the parasite, such as clonal antigenic variation, antigenic diversity, and impairment of dendritic cell maturation by parasitized red blood cells (Casares & Richie, 2009; Chen et al., 1998; Plebanski et al., 1999; Urban et al., 1999). A prospective vaccine will have to overcome all of these.

1.3 The Rationale for a Whole Organism *P. falciparum* Sporozoite-based Vaccine

Hopes that sterile protection against malaria infection can be induced by a whole organism vaccine stem from studies demonstrating that immunization by bites of mosquitoes with radiation-attenuated (>120 Gray units) *Plasmodium falciparum* sporozoites (PfSPZ) in their salivary glands can induce full protection of considerable longevity in mice and humans lasting for up to 10 months, with some evidence of strain-transcending protection (Clyde, 1990; Clyde, McCarthy, Miller, & Hornick, 1973; Hoffman et al., 2002; Nussenzweig, Vanderberg, Most, & Orton, 1967; Rieckmann, Carson, Beaudoin, Cassells, & Sell, 1974). Irradiation of infectious mosquitoes disrupts the gene expression of sporozoites, which remain capable of hepatocyte invasion but are no longer able to complete liver-stage maturation or progress to the pathogenic blood stage (Mellouk, Lunel, Sedegah, Beaudoin, & Druilhe, 1990). Infection of human subjects with irradiated sporozoites thus exposes them to liver-stage antigens and generates pre-erythrocytic immunity. However, the requirement of a minimum of 1,000 bites by irradiated mosquitoes during five or more immunization sessions in order to successfully induce sterile immunity in humans precludes this method for routine immunization (Hoffman et al., 2002).

1.4 The Product PfSPZ Vaccine

Over the last decade, Sanaria, Inc. (Rockville, MD) has developed a novel manufacturing process for obtaining aseptic, purified, cryopreserved sporozoites from the NF54 isolate of *P. falciparum*. PfSPZ are produced by raising adult *A. stephensi* mosquitoes aseptically and having them feed on blood cultures containing aseptic *P. falciparum* gametocytes. Within *P. falciparum* -infected mosquitoes, these *P. falciparum* gametocytes develop into sporozoites. To prepare radiation-attenuated PfSPZ, mosquitoes are irradiated at this stage. This product is named 'PfSPZ Vaccine' (Epstein et al., 2011; Hoffman et al., 2010). The salivary glands are subsequently removed by dissection and triturated to release the sporozoites. The sporozoites undergo several purification steps, are counted, and cryopreserved at a specified concentration. This process is in compliance with cGMPs and regulatory requirements for production of high-quality PfSPZ (for more detailed information, see **Section 5.1** of this protocol).

1.4.1 Rationale for Intravenous Administration of PfSPZ Vaccine

Several animal studies indicate that the immunogenicity and protective efficacy of radiation-attenuated PfSPZ depends to a large extent on the amount of sporozoites reaching the liver which can be influenced by the route of immunization. Compared to subcutaneous (SC) or intradermal (ID) administration, intravenous (IV) immunization using PfSPZ Vaccine induced a significantly higher frequency of PfSPZ-specific CD8+ IFN-γ producing T cells in the liver (demonstrated in nonhuman primates and mice), and conferred protection (tested in mice) (Epstein et al., 2011). Further, protection in IV-vaccinated mice was associated with a 30-fold higher parasite liver load compared to the ID regimen, indicating a high number of sporozoites reaching the liver is a prerequisite for the development of protective immune responses, and that this can be achieved

more easily with IV administration of sporozoites (Nganou-Makamdop et al., 2012; Ploemen et al., 2013).

1.4.2 Previous Trials with Radiation Attenuated PfSPZ (PfSPZ Vaccine)

Multiple clinical trials in the US and abroad have used radiation attenuated *Plasmodium falciparum* sporozoites produced by Sanaria, Inc. (PfSPZ Vaccine) delivered by DVI. Studies conducted in malaria-exposed populations are summarized here briefly.

1.4.3 Results from Malaria Exposed Populations

Four trials in African adults have been completed to date: the first in minimally malaria-exposed Tanzanian adults (clinicaltrials.gov: NCT02132299), the second in heavily malaria-exposed Malian adults (NIAID Protocol 14-I-N010; clinicaltrials.gov: NCT01988636), and the third, in moderately to heavily malaria-exposed Equatoguinean adults (clinicaltrials.gov: NCT02418962). The fourth study in minimally malaria-exposed Tanzanian adults and children as young as six months (ClinicalTrials.gov Identifier: NCT02613520) has also been completed. There are four additional ongoing studies in various African countries evaluating the safety, immunogenicity and efficacy of various doses and administration regimens of PfSPZ Vaccine. Below is a summary of the four completed studies in malaria-endemic countries to date.

1.4.3.1 Mali (NIAID Protocol 14-I-N010)

In the trial in Mali (14-I-N010 conducted by the NIAID Laboratory of Malaria Immunology and Vaccinology [LMIV] and the Malaria Research and Training Center, University of Bamako [MRTC]), healthy Malian adults aged 18 to 35 were immunized with five doses of 2.7×10^5 PfSPZ (total of 13.5×10^5 PfSPZ) or normal saline (placebo) in 2014 (Sissoko et al., 2017). Eighty-eight healthy malaria-exposed Malian adults (44 PfSPZ Vaccine; 44 Placebo) received five doses of vaccine and 86 (42 PfSPZ Vaccine; 44 Placebo) were followed actively every two weeks for up to 24 weeks post-vaccination #5. A total number of 502 immunizations via DVI were administered.

Vaccinations were well-tolerated overall, with no serious adverse events (SAEs) reported during the course of the study. Most study subjects reported no local or systemic reactogenicity following vaccination. Only Grade 1 (mild) local or systemic reactogenicity were reported. There was no local reactogenicity reported in the PfSPZ Vaccine versus four (9%) of the 47 placebo subjects reporting local injection site pain. There were very few subjects who reported any systemic reactogenicity following vaccination (7% in PfSPZ Vaccine; 9% in the placebo; **Table 1**). The most commonly reported solicited systemic adverse event (AE) in both the PfSPZ Vaccine group and the placebo group was headache, followed by fatigue, fever, and myalgia (**Table 1**). No significant differences in local or systemic reactogenicity between PfSPZ Vaccine and placebo recipients were noted (Sissoko et al., 2017).

Table 1: Maximum Local and Systemic Reactogenicity

	Pilot safety cohort (PfSPZ Vaccine; n=12)	Main cohort	
		PfSPZ Vaccine (n=46)	Placebo (n=47)
Local symptoms			
Pain or tenderness			
Grade 1	0	0	4 (9%)
Swelling or redness or induration			
Grade 1	0	0	0
Any local symptom			
Grade 1	0	0	4 (9%)
Systemic symptoms			
Fever or feverish			
Grade 1	1 (8%)	0	1 (2%)
Nausea			
Grade 1	0	0	0
Diarrhoea			
Grade 1	0	0	0
Headache			
Grade 1	3 (25%)	3 (7%)	4 (9%)
Fatigue			
Grade 1	1 (8%)	0	1 (2%)
Myalgia			
Grade 1	0	1 (2%)	1 (2%)
Urticaria			
Grade 1	0	0	0
Any systemic symptom			
Grade 1	3 (25%)	3 (7%)	4 (9%)

Data are n (%), where n represents the number of unique participants with the event. No grade 2–5 adverse events were reported. Solicited adverse events were documented for 7 days after each vaccination. Each vaccine receipt is counted once at worst severity for any local and systemic parameter. Laboratory adverse events are shown in the appendix (appendix p 23). PfSPZ=*Plamodium falciparum* sporozite.

Note: Placebo – Normal Saline

Laboratory variables were closely monitored prior to each vaccination and on Day 3 and 7 following each vaccination. There was no noted increase in reporting of laboratory abnormalities with subsequent vaccinations. Additionally, there were no noted significant differences in laboratory abnormalities between the PfSPZ Vaccine and placebo recipients that were deemed related to vaccination (**Table 2**). Overall, PfSPZ Vaccine was easy to administer by DVI, and was safe and well tolerated.

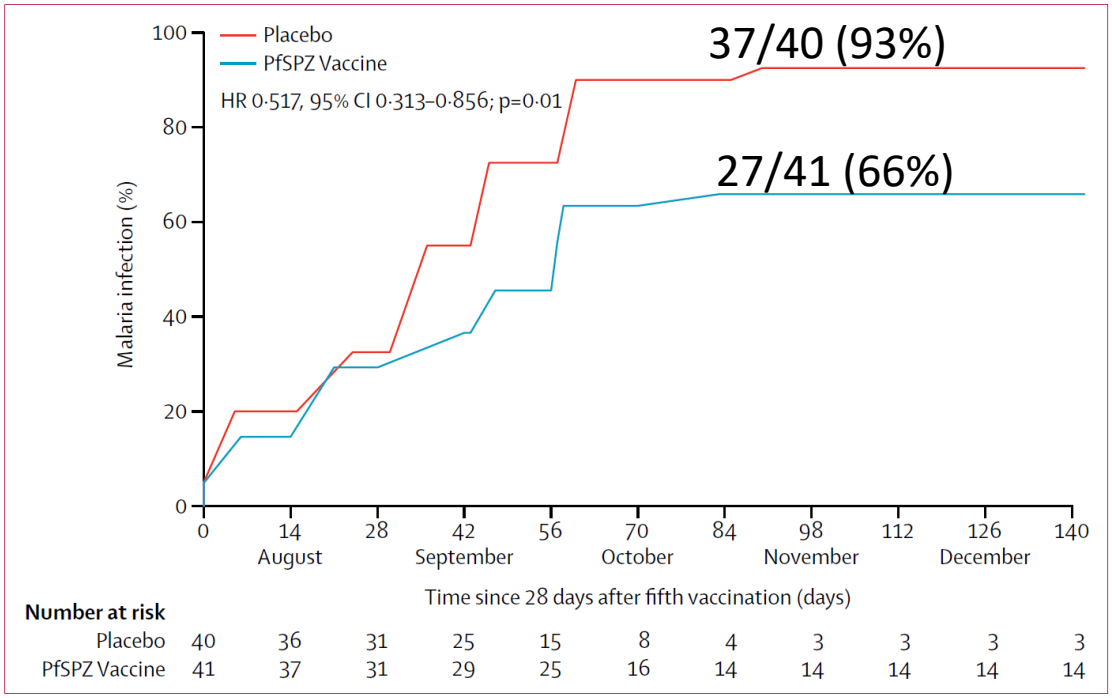
Table 2: Vaccine Related Laboratory Abnormalities

	Pilot Safety Cohort		Main Cohort			
	PfSPZ Vaccine		PfSPZ Vaccine		Placebo (N=47)	
	Total	Related	Total	Related	Total	Related
Laboratory Abnormalities						
Leukopenia						
None	12 (100%)	12 (100%)	39 (85%)	43 (93%)	44 (94%)	45 (96%)
Mild	0 (0%)	0 (0%)	7 (15%)	3 (7%)	3 (6%)	2 (4%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Leukocytosis						
None	12 (100%)	12 (100%)	45 (98%)	46 (100%)	43 (91%)	45 (96%)
Mild	0 (0%)	0 (0%)	1 (2%)	0 (0%)	4 (9%)	2 (4%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Granulocyte count decreased						
None	12 (100%)	12 (100%)	42 (91%)	43 (94%)	45 (96%)	46 (98%)
Mild	0 (0%)	0 (0%)	4 (9%)	3 (7%)	2 (4%)	1 (2%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Thrombocytopenia						
None	11 (92%)	11 (92%)	45 (98%)	46 (100%)	45 (96%)	46 (98%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)
Moderate	1 (8%)	1 (8%)	1 (2%)	0 (0%)	1 (2%)	0 (0%)
Haemoglobin decreased						
None	12 (100%)	12 (100%)	44 (93%)	46 (100%)	45 (96%)	45 (96%)
Mild	0 (0%)	0 (0%)	2 (4%)	0 (0%)	2 (4%)	2 (4%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Blood creatinine increased						
None	12 (100%)	12 (100%)	46 (100%)	46 (100%)	46 (98%)	46 (98%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Alanine aminotransferase (ALT) increased						
None	12 (100%)	12 (100%)	44 (96%)	44 (96%)	47 (100%)	47 (100%)
Mild	0 (0%)	0 (0%)	2 (4%)	2 (4%)	0 (0%)	0 (0%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Data represents total # of individual subjects experiencing AE (% of subjects experiencing AE).
Each vaccine receipt is counted once at worst severity for any laboratory abnormality.
There were no severe laboratory abnormalities.

P. falciparum infections as recorded starting 28 days post vaccination 5 occurred significantly earlier in the control group than the PfSPZ Vaccine group; interval censored log-rank p= 0.01 (VE based on Cox HR, 48.27% 95% CI (14.45, 68.72)). The proportion of individuals with any infection as recorded starting 28 days post vaccination 5, during the malaria season, was 28.8% lower (95% CI 8.2-47.2, p=0.006) in the PfSPZ Vaccine group than the control group. The non-parametric maximum likelihood estimation (MLE) curves, accounting for interval censoring, for the vaccine and placebo groups in the Mali trial are presented in **Figure 1**(Sissoko et al., 2017).

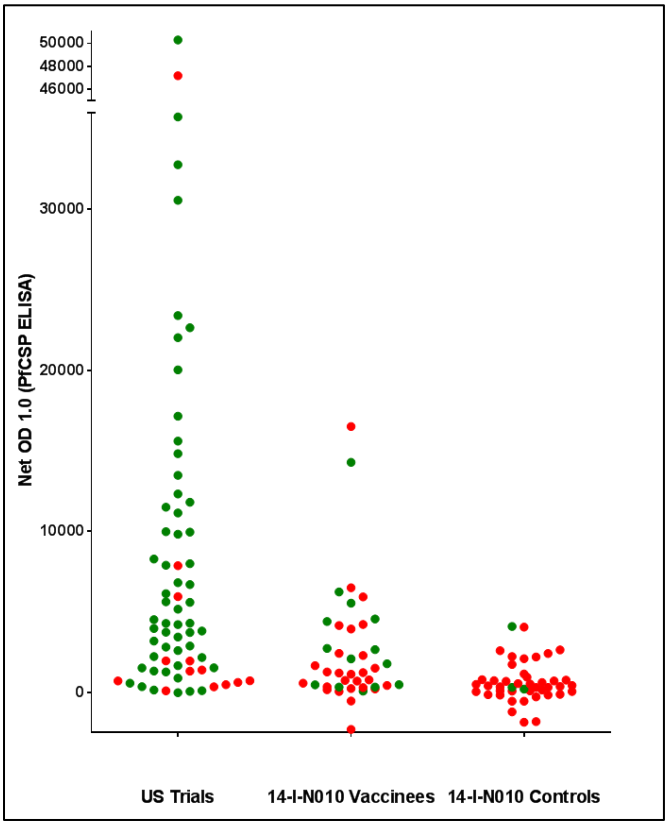
Figure 1: Protective Efficacy: First Positive Blood Smear (BS) Starting 28 Days Post Vaccination #5



In the pilot safety group 7/9 (78%) were blood smear (BS) positive. Protective efficacy was analyzed by time to first positive BS, with Day 0 at 28 days after the fifth vaccination. The inverse survival curves include participants who received all five vaccinations and were evaluable for the primary exploratory efficacy endpoint. Five subjects (1 PfSPZ Vaccine; 4 placebo) were censored from primary efficacy analysis as they had a positive BS prior to 28 days post Vaccination #5.

PfSPZ vaccination induced only modest antibody responses to CSP and antibody levels. Antibody levels were not significantly predictive of infection in the vaccine arm subjects. When results of the *Plasmodium falciparum* circumsporozoite protein (PfCSP) enzyme-linked immunosorbent assay (ELISA) were compared between the USA volunteers and the Tanzanian and Malian volunteers, it was clear that antibody responses to PfSPZ Vaccine were lower in malaria-exposed than in malaria-naive subjects. **Figure 2** shows the PfCSP ELISA results (net OD 1.0) after three doses for volunteers in VRC 314 and WRAIR 2080 (N = 36) who received four or five doses of 2.7×10^5 PfSPZ/dose versus volunteers in 14-I-N010 (N = 41) who received five doses of 2.7×10^5 PfSPZ/dose.

Figure 2: USA vs Mali PfCSP ELISA Results



Controls from 14-I-N010 (N = 44) are also included. Assay was performed on serum drawn following the third vaccine dose. The Malians had significantly lower responses, with many giving negative results (i.e., lower OD 1.0 following the third dose than prior to the first vaccination). Green dots represent volunteers who were protected against CHMI or natural exposure; red dots represent volunteers who were not protected.

1.4.3.2 Tanzania, BSPZV1

When healthy, young males in Tanzania (clinical trial BSPZV1 conducted by the Ifakara Health Institute) received five doses of either 1.35×10^5 PfSPZ Vaccine (total of 6.75×10^5 PfSPZ) or 2.7×10^5 PfSPZ Vaccine (total of 13.5×10^5 PfSPZ)(regimens similar to that which protected 69% and 92% of volunteers in the USA, respectively), and underwent homologous CHMI (by DVI of 3,200 viable *Plasmodium falciparum* sporozoites produced by Sanaria, Inc. [PfSPZ Challenge]) three weeks after the fifth dose, only 4/19 (21%) and 4/20 (20%) were protected. As in Mali, the vaccine was well-tolerated and immune responses were markedly reduced compared to those recorded in malaria-naïve individuals.

1.4.3.3 Mali (NIAID Protocol 16-I-N004)

The phase 1 dose escalating and randomized, placebo-controlled, double-blind study to assess the safety, immunogenicity, and protective efficacy of PfSPZ Vaccine in Mali started in December 2015 and was completed per protocol in January 2017.

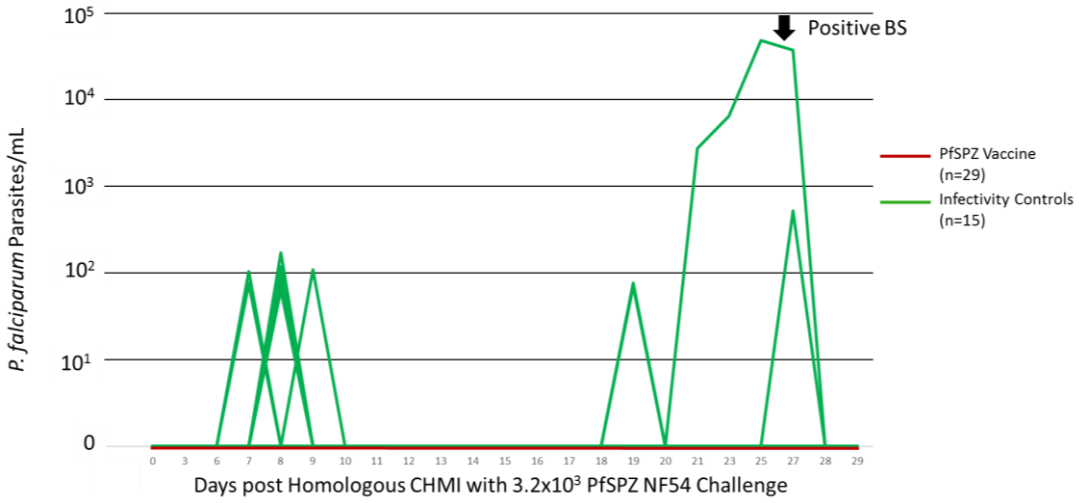
1.4.3.3.1 Pilot Phase

In January 2016, enrollment of the open label dose escalation pilot study (n=40) was completed and demonstrated the safety and tolerability of PfSPZ Vaccine at $4.5\text{x}10^5$, $9\text{x}10^5$, and $1.8\text{x}10^6$.

Overall, PfSPZ Vaccinations were very well tolerated in those receiving $4.5x10^5$ (n=5) and $9x10^5$ (n=5) with few adverse events (AEs) and no Grade 3 AEs or lab abnormalities reported. Subjects then were enrolled and vaccinated in a staggered manner for safety to receive $1.8x10^6$ PfSPZ Vaccine (Arm 1c). Arm 1c was also well tolerated by the majority of subjects; however, there was one subject (randomized to artesunate-amodiaquine [ASAQ] treatment prior to each vaccination) who experienced an asymptomatic Grade 3 elevated ALT following Vaccination #1. This AE, along with three other ALT elevations that developed around Vaccination #3 during the Main Phase of the study, were reviewed at length by the Data Safety Monitoring Board (DSMB), ISM, and Sponsor and are summarized later below. Vaccination #2 and #3 in $1.8x10^6$ PfSPZ Vaccine Pilot arm were completed without significant complication and were well tolerated. Approximately seven weeks prior to homologous CHMI with $3.2x10^3$ PfSPZ Challenge, 29 (96.7%) pilot phase PfSPZ vaccinees who received $1.8x10^6$ PfSPZ Vaccine (Arm 1c) and 15 infectivity controls received a full treatment course ASAQ. All 44 subjects, including 29 PfSPZ Vaccinees (Arm 1c), underwent homologous CHMI about five weeks following PfSPZ Vaccinees' receipt of their third PfSPZ Vaccine. All 44 subjects completed CHMI follow-up. Reported AEs post CHMI were few, with vaccinees reporting five total AEs (4/29 subjects; 13.8%) and infectivity controls eight total AEs (7/15 subjects; 46.7%). All reported AEs were mild to moderate. Only one AE, granulocyte reduction, was deemed related to CHMI. Of the 29 PfSPZ vaccinees, 0/29 vaccinees and 1/15 infectivity controls became BS positive. By quantitative polymerase chain reaction (qPCR) 0/29 vaccinees and 8/15 (53.3%) infectivity controls became positive (**Figure 3**). Most qPCR positive infectivity controls were qPCR positive only for a single timepoint (5/8; 62.5%; **Figure 3**). By qPCR, VE was significant by interval-censored log rank $p<0.001$ for time-to-infection, and VE was 100% ($p<0.001$, 95% CI 73-100%) by proportional analysis.

Twenty-seven (90%) subjects continued in the study to receive a final fourth booster dose (13 weeks post Vaccination #3; 8 weeks post CHMI) of PfSPZ Vaccine in parallel with the main cohort's third vaccination. Of those 27 subjects, 18 (66.7%) became infected during the malaria transmission season.

Figure 3: Protective Efficacy of 18x105 PfSPZ Vaccine at 0, 8, 16 weeks against Homologous PfSPZ Challenge



1.4.3.3.2 **Main Phase**

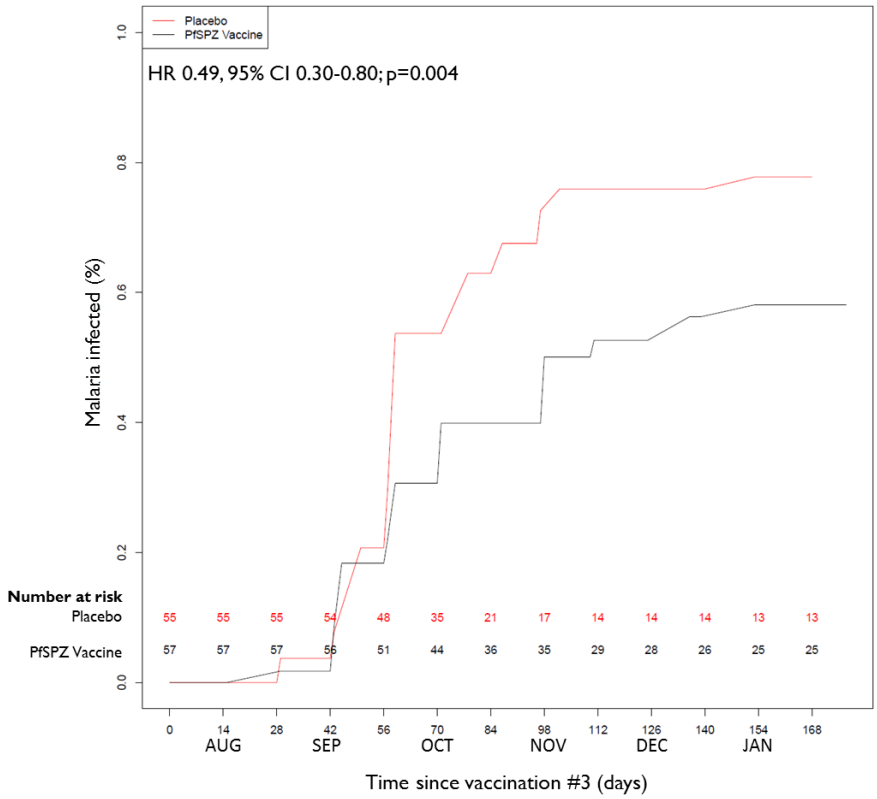
Following review of the safety data from the Pilot Phase, 120 subjects were randomized into the double blind, placebo-controlled phase of the study in Mar/Apr 2016 to receive $1.8x10^6$ PfSPZ Vaccine or normal saline at 0, 8, 16 weeks during the dry season (Mar to Jul). All participants received ASAQ to eliminate Pf before first and last vaccination. During the malaria transmission season (Aug-Dec 2016), volunteers were examined and BS obtained every 2 weeks for 24 weeks in total; the primary efficacy endpoint was detection of first positive BS following third vaccination. 60 subjects received at least one dose of PfSPZ Vaccine and 60 subjects received at least one dose of placebo. 57/60 (95%) PfSPZ Vaccine subjects received all three doses and 55/60 (91.7%) normal saline placebo subjects received all three doses. Scheduled unblinding of the trial was completed in January 2017.

PfSPZ vaccinations were safe and well tolerated except as noted prior, there were three additional unanticipated significantly elevated transaminases (Grade 3 to 4) in three Main Phase participants (1 PfSPZ Vaccine, 2 placebo) at varying stages post vaccination and ASAQ (14 to 65 days post ASAQ). Including the ALT liver enzyme derangement seen in the Pilot Phase subject, all four subjects were asymptomatic at presentation with no associated agranulocytosis. All laboratory abnormalities resolved without sequelae (duration 169 to 214 days). At the time of the laboratory abnormalities the study was blinded, and the unblinded data was reviewed by the DSMB and submitted to the Food and Drug Administration (FDA) for review. Testing for potential of other etiologies, through imaging, expanded laboratory testing, and serology, identified no other possible contributing causes, except a traditional medicine provided to the first subject presenting with liver enzymes derangements. Serum antibodies to AQ are pending at this time.

Overall, there was no significant differences in local or systemic AEs or laboratory abnormalities between PfSPZ Vaccine and placebo groups.

55 subjects in the PfSPZ Vaccine group and 54 subjects from the placebo group were evaluable for per protocol analysis. Of these participants, 42 (77.8%) from the placebo group and 32 (58.1%) from the vaccine group developed Pf infection (**Figure 4**). Per protocol, VE was 51% (p=0.004, 95% CI 20-70) by time-to-infection analysis (ITT 39%, p=0.033) and 24% (p=0.031, 95% CI 2-41) by proportional analysis (ITT 22%, p= 0.041).

Figure 4: Protective Efficacy of PfSPZ Vaccine Against Naturally Occurring Infection



Protective efficacy was analyzed by time to first positive blood smear (BS), with Day 0 starting immediately after the third and final vaccination. The survival curves include participants who received all three vaccinations.

1.4.3.4 Tanzania, BSPZV2

A follow up expanded study was conducted in Tanzania (clinical trial BSPZV2 conducted by the Ifakara Health Institute). This double-blind randomized placebo-controlled study enrolled children from six months of age to adults 45 years of age to assess the safety and immunogenicity of PfSPZ Vaccine. The participants were divided in various 3-dose regimens starting from 2.7×10^5 PfSPZ Vaccine to $1.8\text{x}10^6$ PfSPZ Vaccine. In addition, adult participants underwent homologous CHMI with PfSPZ Challenge (live NF54 *P. falciparum* parasites). The study results showed that the regimen was safe and tolerable. One adult participant who received $1.8\text{x}10^6$ PfSPZ Vaccine experienced 3 Grade 1 solicited local AEs that were considered possibly, probably or definitely related to vaccination. One teenage participant experienced a total of 15, mostly Grade 1 (maximum Grade 2) solicited systemic AEs which occurred after different vaccinations that were considered possibly, probably or definitely related to vaccination. These symptoms were transient and without sequalae. Safety results during vaccination period are summarized in **Table 3**.

Table 3: Summary of clinical adverse events of BSPZV2 during vaccination

Number of Volunteers Immunized = 93	Vaccine N=63		Placebo N=30	
	All Adverse Events	Possibly, Probably or Definitely Related Adverse Events	All Adverse Events	Possibly, Probably or Definitely Related Adverse Events
Volunteers with at least one solicited AE within 7 days of immunization [n (%)]	2 (3.2%)	2 (3.2%)	0 (0.0%)	0 (0.0%)
Total # solicited AEs [n (maximum severity grade)]	19 (Grade 2=Moderate)	18 (Grade 2=Moderate)	0 (NA)	0 (NA)
Volunteers with a solicited Grade 3 AE [n (%)]	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Volunteers with at least one solicited local AE	1 (1.6%)	1 (1.6%)	0 (0.0%)	0 (0.0%)
Total # local AEs	3 (Grade 1=Mild)	3 (Grade 1=Mild)	0 (NA)	0 (NA)
Average # local AEs/volunteers experiencing local AEs	3.00	3.00	NA	NA
Volunteers with at least one solicited systemic AE	1 (1.6%)	1 (1.6%)	0 (0.0%)	0 (0.0%)
Total # systemic AEs	16 (Grade 2=Moderate)	15 (Grade 2=Moderate)	0 (NA)	0 (NA)
Average # systemic AEs/volunteers experiencing systemic AEs	16.00	15.00	NA	NA
Volunteers with at least one unsolicited AE within 28 days of immunization [n (%)]	20 (31.7%)	0 (0.0%)	10 (33.3%)	1 (3.3%)
Total # unsolicited AEs within 28 days of immunization [n (maximum severity grade)]	30 (Grade 2=Moderate)	0 (NA)	12 (Grade 1=Mild)	2 (Grade 1=Mild)
Volunteers with an unsolicited Grade 3 AE [n (%)]	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Volunteers experiencing an SAE [n (%)]	1 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total # of SAEs [n (maximum severity grade)]	1 (Unknown)	0 (NA)	0 (NA)	0 (NA)

Efficacy results: This trial compared $9.0x10^5$ PfSPZ administered at 0, 8 and 16 weeks to twice that dose, $1.8x10^6$ PfSPZ, also administered at 0, 8 and 16 weeks. Half the lower dose group (3 subjects) underwent homologous CHMI at 3 weeks and the other half (3 subjects) at 11 weeks. All 6/6 (**100%**) were protected. The higher dose group (six subjects) underwent homologous CHMI at 7 weeks and 2/6 (**33%**) were protected. It appeared that doubling the dose may have resulted in a significant fall in VE.

1.5 Rationale for decreasing the dose of PfSPZ Vaccine from $1.8x10^6$ to $9x10^5$ in a three-dose regimen

The hypothesis underlying the sequence of studies done with PfSPZ Vaccine (described above and others) was that higher doses of PfSPZ would lead to improved efficacy. It was argued that increasing the quantity of the immunogen should increase the antigenic stimulus and should also broaden the repertoire of immune responses to include less highly expressed proteins, improving VE. The expectations under this hypothesis: (1) long term CHMI (conducted at 24 weeks or longer after immunization) should be more stringent than short term CHMI (conducted 3-10 weeks after immunization) due to the fact that immune responses likely wane over time; and (2) the use of heterologous parasites (different strain than the vaccine) for CHMI should be more stringent than the use of homologous parasites (same strain as the vaccine). Therefore, the goal was to investigate and determine the most stringent challenge – long-term protection against CHMI performed with an antigenically divergent parasite – would most closely mimic field studies, where naturally transmitted parasites should all be heterologous to the vaccine strain.

Findings of the 12 studies have shown that the threshold dose hypothesis is only partially true. The initial studies did confirm that VE increased with the dose of PfSPZ Vaccine administered. There was a clear dose response seen in several cases. For example, VE against homologous CHMI

conducted 3 weeks after a 3-dose regimen rose from 33% (3/9) in VRC314 to 87% (13/15) in WRAIR 2080 as the dose of PfSPZ administered was increased from 2.7×10^5 to 4.5×10^5 PfSPZ (Epstein et al., 2017; Ishizuka et al., 2016) VRC314 also tested a higher dose, $9.0x10^5$ PfSPZ, administered at 0, 8 and 16 weeks, and VE was 64% (9/14) against a heterologous CHMI conducted at 17-20 weeks. VE was still high, despite a considerably more stringent CHMI than 3 weeks. However, as individual doses were increased above about $9.0x10^5$ PfSPZ Vaccine, further improvements were not generally seen. In addition to the BSPZV2 study described above (**Section 1.4.3.4**), there have been several examples:

1. Warfighter 2 trial (malaria-naïve population, USA NCT02601716): This trial compared four different regimens for protection against heterologous CHMI performed at 3 or 6 months. $9.0x10^5$ PfSPZ administered at 0, 8 and 16 weeks protected 3/15 (**20%**) research subjects (total dose administered $2.7x10^6$ PfSPZ), while doubling the dose to $1.8x10^6$ PfSPZ administered at 0, 8 and 16 weeks protected 3/13 (**23%**) subjects (total dose administered $5.4x10^6$ PfSPZ) – no improvement. On the other hand, the most protective regimen used doses of only $4.5x10^5$ PfSPZ, administered Days 0, 2, 4, 6 and week 16, protecting 6/15 (40%) research subjects against a stringent heterologous CHMI (total dose administered $2.25x10^6$ PfSPZ). This latter dose likely improved VE through the use of the condensed, 4-dose prime, indicating that dose number and timing were critical.

2. MAVACHE trial (malaria-naïve population, Germany NCT02704533): In addition to testing a 3-dose condensed regimen of $9.0x10^5$ PfSPZ (total of $2.7x10^6$ PfSPZ) at 0, 1 and 4 weeks which protected 5/5 **(100%)** subjects against homologous CHMI 3 weeks after last vaccination. This trial also compared two 2-dose condensed regimens: $1.8x10^6$ PfSPZ administered at 0 and 1 week (total of $3.6x10^6$ PfSPZ) protected 4/6 (**67%**) research subjects against homologous CHMI conducted at 3 weeks, while increasing the dose to $2.7x10^6$ PfSPZ administered at 0 and 1 week (total of $5.4x10^6$ PfSPZ) protected 3/6 (**50%**) research subjects. Thus, no dose response was observed, and it appeared that the higher dose may have been less protective.

Although the studies described above have been conducted in malaria naïve subjects, there is some evidence that these results can be compared to those seen in field studies in malaria experienced. Increasing the dose of PfSPZ Vaccine past a particular threshold does not seem to improve VE, total of $1.35x10^6$ PfSPZ Vaccine in NIAID protocol #14-I-N010 versus a total of $5.4x10^6$ PfSPZ Vaccine in NIAID protocol #16-I-N004, **Sections 1.4.3.1 and 1.4.3.3**.

As a result of these combined studies, it is the opinion of the sponsor, Sanaria Inc., that $9.0x10^5$ PfSPZ Vaccine per dose in a 3-dose regimen is an optimal dose in both malaria-naïve and malaria-exposed populations. It induced 100% protection in the MAVACHE trial administered in a condensed regimen (0, 1 and 4 weeks) and it provided 100% protection in Tanzania in BSPV2 trial when a higher dose provided only 33% protection, when administered at 0, 8 and 16 weeks. This dose will be administered in this proposed study.

1.6 Rationale for assessing various vaccination schedules

A number of PfSPZ Vaccine studies are ongoing to determine a highly effective vaccine schedule. There is ample evidence that the timing of each vaccine plays an important role in the development of protective immunity. A recent study in malaria naïve subjects, MAVACHE study described in **Section 1.5**, suggests that a condensed regimen of 0, 1 and 4 weeks injections may lead to equal or higher protective efficacy.

This condensed vaccine regimen was also found to be safe and well tolerated. Among the six subjects, during the time from first injection and prior to CHMI, there was a total of 82 AEs, 74% of which were determined related to the study. There was a total of 3 Grade 3 AEs in two subjects which were also thought to be related to the study. The first subject had lymphopenia one day after 3rd vaccination. The second subject had 2/3 Grade 3 AEs; lymphopenia 12 days after 2nd vaccination and fever two days after 3rd vaccination. No SAE was observed.

The safety profile and protective efficacy of this regimen are encouraging. Since our goal at LMIV is to understand the immune response to vaccine candidates, this proposed study will also evaluate a condensed regimen of 0, 1, and 4 weeks in a field setting to assess whether this regimen is safe and tolerable in this population. We will also evaluate the protective efficacy observed with this condensed regimen and explore the immune response to vaccination.

1.7 Rationale for administering the booster dose (4th vaccination)

The main phase of the study was designed to assess the safety and immunogenicity of two different vaccine regimens, an established regimen of 0, 8 and 16 weeks and a condensed regimen of 0, 1, and 4 weeks. The study started in June 2018 and the 3rd vaccination in all *Arms* was administered in September 2018. Currently, the participants are nearing the end of the malaria follow up phase during the first transmission season. The current plan is to extend the study by evaluating the safety and immunogenicity in the second transmission season after administration of a booster vaccination.

1.7.1 Safety

Thus far, the vaccine regimen has been safe and well tolerated. There have been no serious adverse events (SAEs) or Grade 4 AEs. As expected, there are higher numbers of AEs in standard longer regimen *Arm* (*Arm 1/3a*) as this group has been enrolled and in follow up for a longer period of time than the condensed regimen *Arm* (*Arm 2/3b*). However, as demonstrated in **Table 4**, the proportions of AEs are similar between the two regimens indicating that administering PfSPZ Vaccine within a short period of time does not result in higher numbers of AEs compared to the standard longer regimen. All AEs were transient and have resolved without sequelae.

Grade 1 AEs

There have been 744 Grade 1 AEs to date. Lab abnormalities account for 153 (20.5% of total; 97 in *Arm 1/3a* and 56 in *Arm 2/3b*). The majority have been absolute neutrophil count decreases (30 in *Arm 1/3a*; 22 in *Arm 2/3b*), white blood cell count decreases (34 in *Arm 1/3a*; 23 in *Arm 2/3b*), and increased blood creatinine (20 in *Arm 1/3a*; 6 in *Arm 2/3b*). Most of the clinical AEs have been headache, injection site pain, malaria, and upper respiratory like illnesses.

Grade 2 AEs
There have been 71 total AEs to date (46 in *Arm 1/3a*; 25 in *Arm 2/3b*). Lab abnormalities account for 43 (60.5% of total; 29 in *Arm 1/3a* and 14 in *Arm 2/3b*). Of the 43 lab abnormalities, 23 have been from absolute neutrophil count decreases, with 16/23 occurring in *Arm 1/3a*, 5 were determined to be related to the study. 7 episodes have occurred in *Arm 2/3b*, 3 were determined to be related to the study. There have been 28 variable clinical AEs, most common being hypertension (4/17 in *Arm 1/3a*; 2/11 in *Arm 2/3b*) and malaria cases.

Grade 3 AEs
There have been 16 total AEs to date (10 in Arm 1/3a; 6 in Arm 2/3b). There was only 1 AE of decreased platelet count (in Arm 1/3a) thought possibly related to the vaccine due to timing. There was a total of 8 Grade 3 malaria AEs, 4 in each arm. These participants presented with Grade 3 fever but were otherwise in stable condition.

Table 4: Summary of AEs to date

	Arm 1/3a (n=105)	Arm 2/3b (n=105)	TOTAL
Clinical AEs			
Grade 1	397 (72%)	194 (69%)	591 (71%)
Grade 2	17 (3%)	11 (4%)	28 (3.4%)
Grade 3	9 (1.6%)	5 (1.8%)	16 (1.9%)
Lab abnormalities			
Grade 1	97 (18%)	56 (20%)	153 (18.4%
Grade 2	29 (5.3%)	14 (5%)	43 (5.2%)
Grade 3	1 (0.2%)	1 (0.3%)	2 (0.2%)
TOTAL	550 (66%)	281 (34%)	831 (100%)

X(Y%): Number of AEs (Percentage of the Total combined AEs in Arm)

1.7.2 Malaria infection rate

Vaccine efficacy started immediately after the third vaccination. Participants have been evaluated for malaria infection using blood smears every two weeks. The study is currently still blinded, but in general, there appears to be no significant differences between the study arms in the number of positive blood smears, clinical malaria and the unique individuals experiencing these events (see **Table 5** below).

Table 5: Current rates* of malaria infection after vaccination #3

Study Arms (# of participants who received vaccination #3)	Positive *P. falciparum* Blood smears (Unique individuals)	Clinical Malaria (Unique Individuals)
1/3a (n=101)	87 (53)	45 (35)
2/3b (n=103)	77 (51)	40 (36)

*September 2018 to Early February 2019

1.7.3 Booster dose administration

A previous PfSPZ Vaccine study (ClinicalTrials.gov Identifier: NCT02627456) was conducted by LMIV to evaluate whether the vaccine efficacy observed in the first transmission season after the primary vaccine series extends to a second transmission season. Although analysis is ongoing, the preliminary results indicate that the rates of malaria infection were similar between the control and vaccinated groups, indicating that the immunity against malaria may have waned with time. Administering a booster vaccine dose is one of the ways we may extend vaccine efficacy into subsequent seasons.

In order to explore the effect of boosting, all participants who received the full primary series (3 vaccinations) in the previous year will be invited to receive a 4th dose of 9.0x10^5 PfSPZ, same dose as used in the first three vaccinations. Participants enrolled in the control *Arms* will receive an injection with normal saline. Understanding the role of boosting in this model of malaria vaccine is essential to determine whether endemic populations who have received a primary vaccination series could benefit from receiving a booster dose only prior to each transmission season. In addition in the future, vaccinated individuals could receive a booster vaccination at the time when they are more susceptible to malaria such as during pregnancy.

2 STUDY OBJECTIVES

2.1 Primary Objective

Safety:

1. Assess safety and tolerability of PfSPZ Vaccine primary series in healthy Malian adults when given at 0, 8 and 16 weeks and at 0, 1 and 4 weeks (Main phase)
2. Assess safety and tolerability of PfSPZ Vaccine booster dose (4th dose) in healthy Malian adults (Booster phase)

2.2 Secondary Objective

Protective Efficacy:

1. Assess the efficacy PfSPZ Vaccine primary series in healthy Malian adults when given at 0, 8 and 16 weeks and at 0, 1, and 4 weeks (Main phase).
2. Assess the efficacy of PfSPZ Vaccine booster dose (4th dose) in healthy Malian adults (Booster phase)

2.3 Exploratory Objectives

Immunogenicity

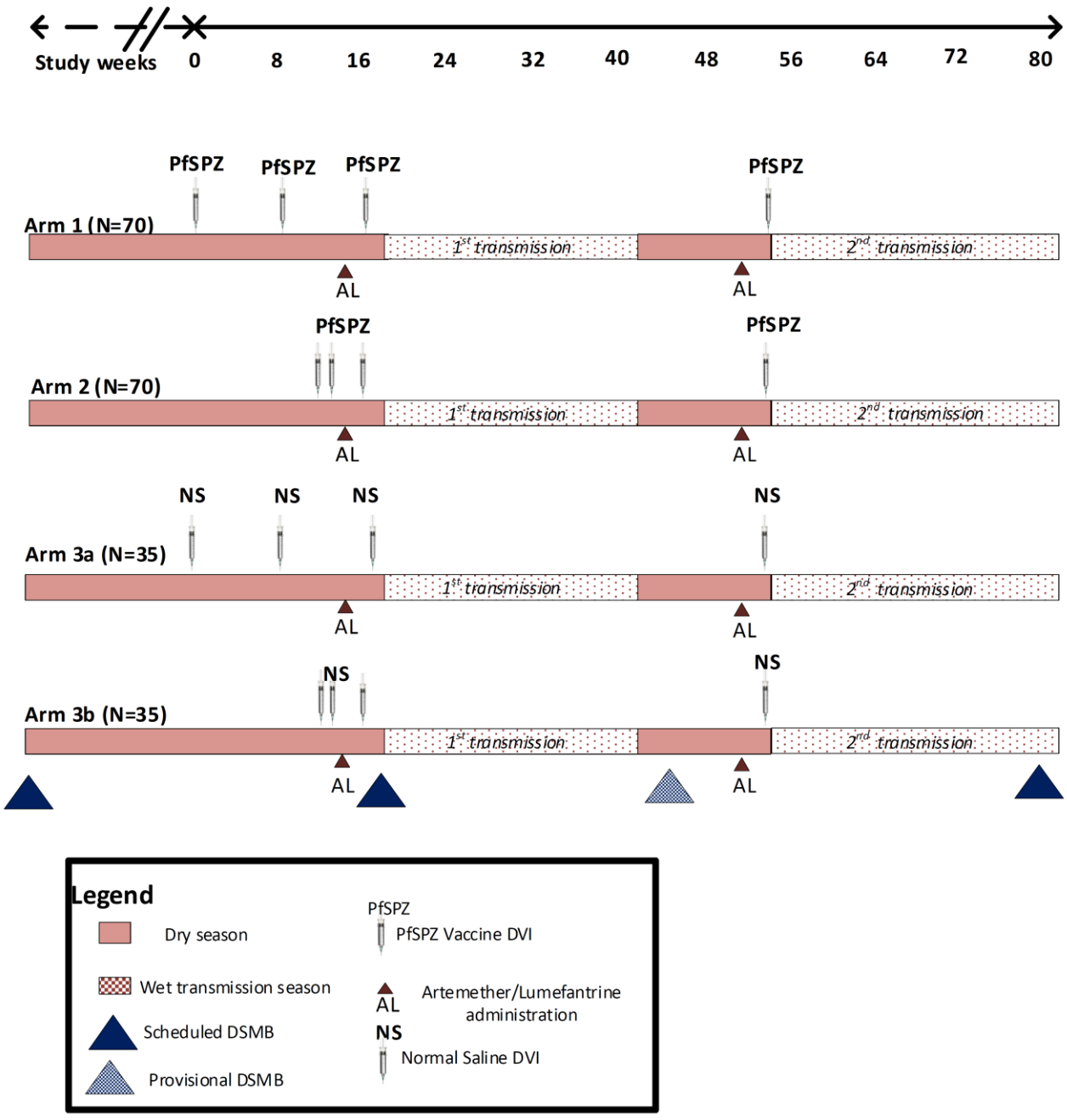
- Characterize and compare host immune responses to malarial antigens, and host proteomic profiles and transcriptomes in African adults prior to and after vaccination with PfSPZ Vaccine and between uninfected and infected subjects
- Explore the impact of PfSPZ Vaccine on the genotype and transcriptome profile of parasites isolated from study subjects.

3 STUDY DESIGN

This study will enroll healthy Malian adults between 18 and 35 years of age to participate in a randomized double blind, placebo-controlled study to assess the safety, immunogenicity and protective efficacy of boosting during the second transmission season. Participants will be immunized with a 3-dose series of 9 x10^5 PfSPZ Vaccine (cryopreserved radiation attenuated *P. falciparum* sporozoites via DVI) at 0, 8 and 16 weeks interval or 0, 1 and 4 weeks starting during the dry season (see **Figure 5**). Vaccinated subjects and controls will then be assessed for malaria infection during the ensuing malaria transmission season. Volunteers will be randomized into four arms.Arms 1 and 3a will be randomized first in a 2:1 ratio. Arms 2 and 3b will be randomized into a 2:1 ratio a few weeks later.

At the completion of the follow up phase after the primary series, participants who received all three vaccine doses and are still enrolled in the study will be invited to continue participation in the study to receive a booster vaccine dose. This 4th dose will be administered prior to the beginning of the following transmission season, approximately10 months post the 3rd vaccination. Those enrolled in the vaccine arms (*Arms 1* and *2*) will receive one injection of 9 x10^5 PfSPZ while those participants enrolled in the control arms (*Arms 3a* and *3b*) will receive one injection of normal saline. The study will be maintained blinded as the participants transition from the main phase to the booster phase. Vaccinated subjects and controls will then be assessed for malaria infection during the ensuing malaria transmission season.

Figure 5: Study Schema



3.1 Study Groups

Arm 1: (n=70) will receive 3 doses of PfSPZ Vaccine (9 x10^5) via DVI at 0, 8, and 16 weeks in the main phase, booster dose at 38 weeks post 3rd vaccination.

Arm 2: (n=70) will receive 3 doses of PfSPZ Vaccine (9 x10^5) via DVI at 0, 1 and 4 weeks in the main phase, booster dose at 38 weeks post 3rd vaccination.

Arm 3a: (n=35): Will be the control for Arm 1. Volunteers will receive 3 doses of placebo saline injection via DVI at 0, 8 and 16 weeks in the main phase, booster dose at 38 weeks post 3rd vaccination.

Arm 3b: (n=35): Will be the control for Arm 2. Volunteers will receive 3 doses of placebo saline injection via DVI at 0, 1 and 4 weeks in the main phase, booster dose at 38 weeks post 3rd vaccination.

All injections will be administered by DVI. All volunteers will receive antimalarial treatment with artemether/lumefantrine (AL) two weeks prior to 3rd injection. Post 3rd injection, participants will be followed through the rainy transmission season, approximately six months. Participants will be monitored for safety, immunogenicity, malaria infection and disease during the follow-up period.

Participants enrolled in the booster phase of the study, at the end of the prior transmission season, will also receive antimalaria treatment with artemether/lumefantrine (AL) at least two weeks prior to booster injection (4th injection overall). Post 4th injection, participants will be followed through the rainy transmission season, approximately six months. Participants will be monitored for safety, immunogenicity, malaria infection and disease during the follow-up period.

4 STUDY ENDPOINTS

4.1 Primary Endpoints

Safety:

1. Incidence of local and systemic adverse events graded by severity occurring within 7 days after each vaccine administration. (Main phase).
2. Incidence of local and systemic adverse events (AEs) graded by severity occurring within 7 days after each vaccine administration (Booster phase).

4.2 Secondary Endpoints

Protective Efficacy:

1. *P. falciparum* blood stage infection defined as time to first positive blood smear (detection of at least two *P. falciparum* parasites by microscopic examination of 0.5 µL) starting immediately following 3rd injection. (Main Phase)
2. *P. falciparum* blood stage infection defined as time to first positive blood smear (detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL) starting immediately following 4th injection (Booster Phase).

4.3 Exploratory Endpoints

Immunogenicity:

- Measurement and comparison of various immunological parameters:
  - in the same subject prior to and after vaccination
  - between parasitemic and aparasitemic subjects
- Determination of the genotype of parasites isolated from study subjects, using microsatellite typing, sequencing, and/or SNP chip assays.

4.4 Sample Size and Estimated Duration of the Study

A total of 210 volunteers will be enrolled/randomized in this trial, 70 subjects in each arm vaccine arms (Arm 1 and 2) and 35 subjects in each control arms (Arm 3a and 3b). A total of 140 subjects will receive PfSPZ Vaccine and 70 will only receive placebo.

Definitions for the purpose of this study:

- **Screened** – subjects will receive a study screening number when the informed consent is signed and will either be determined as "eligible" or "screen failure" as noted below.
  - Screening may be completed over the course of multiple visits.
  - Screening, in most cases, will occur within 56 days prior to administration of vaccine #1.
    - If the screening visit is >56 days administration of vaccine #, then an updated medical review and laboratory testing (CBC, ALT, Creatinine, ECG, Urinalysis, Pregnancy test (for females) will be completed to determine eligibility for enrollment.
    - **Note:** HIV, Hepatitis B, Hepatitis C and ECG are to be repeated during booster phase screening ***ONLY IF*** clinically indicated
- **Eligible -** subjects will be considered eligible to enroll once they have completed the screening procedures and have **met all inclusion** and **not met exclusion** criteria.
- **Screen Failure** – subjects are considered screen failures when they meet one of the following criteria after signing consent:
  - Screening results reveal that the subject is ineligible.
  - Subject withdraws consent before being vaccinated and/or randomized.
  - Subject cannot present to clinic for enrollment prior to study completing enrollment.
  - Subject is determined eligible for enrollment after study completes enrollment.
- **Randomized** – the subjects are considered randomized when they meet the following criteria:
  - Meet eligibility criteria.

- Study identification number (ID) assigned.
- Randomization number assigned.

- **Enrolled** – In the main phase, subject will be considered enrolled beginning with the collection of baseline labs on study Day -14.

  – In the booster phase, subject will be considered enrolled beginning with the receipt of artemether/lumefantrine prior to receiving 4th vaccination

  - Subjects enrolled in main phase but have not received the first vaccination may be replaced up to the accrual ceiling set for the study.

- **Discontinued/Withdrawn** – subjects are considered discontinued when they meet one or more of the following criteria:
  - Subject withdraws consent after being vaccinated.
  - Subject is withdrawn by the Principal Investigator (PI)/Sponsor after being vaccinated.

- **Completed** – subjects are considered completed when they complete the final study visit. ***Note***: Subjects who do not enroll in the booster phase will be considered completed if they complete the main phase final visit; Study day 295 (*Arms 1 &3a*) or 211 (*Arms 2&3b*)

5 STUDY AGENTS

5.1 PfSPZ Vaccine

The vaccine referred to as PfSPZ Vaccine contains aseptic, purified, vialed, cryopreserved, radiation attenuated NF54 *P. falciparum* sporozoites (PfSPZ) produced by Sanaria Inc. PfSPZ Vaccine is manufactured in compliance with Good Manufacturing Practice (GMP) regulations (21 Code of Federal Regulations [CFR] 211), that is described in detail in Investigational New Drug (IND) 13969. Manufacture of PfSPZ Vaccine is performed in Sanaria's Clinical Manufacturing Facility (CMF) in Rockville, Maryland, USA. The PfSPZ Vaccine manufacture is an aseptic process that includes in process testing according to USP<71> sterility testing. In brief, manufacture includes disinfection of mosquito eggs performed by exposure to chemical agents. Disinfected eggs are inoculated into vented flasks containing growth medium. The eggs hatch and develop into pupae, which are transferred to an adult mosquito container where the adult mosquitoes emerge. *In vitro* culture of P. falciparum parasites is initiated from a working cell bank (WCB) vial of *P. falciparum* isolate NF54, which is described in detail in Biologics Master File BMF 13489. The asexual parasite stages are induced to produce gametocytes. The adult mosquitoes are fed *P. falciparum* gametocyte-infected blood in a high-security insectary in Rockville, Maryland, USA. Infected adult mosquitoes are maintained and sporozoites migrate to the salivary glands in two weeks from the time of infectious feed. The PfSPZ in the mosquito salivary glands are attenuated by irradiation at 150 Gy. The salivary glands from the *P. falciparum* sporozoite infected mosquitoes are harvested by manual dissection. Salivary glands

are then triturated to release the PfSPZ that are then purified, counted, and, at a specified concentration, cryopreserved. Cryopreservation commences with the addition of cryoprotective additives to the PfSPZ bulk product to produce the PfSPZ Vaccine final product. The final product is dispensed into screw-cap vials that are stored in liquid nitrogen vapor phase (LNVP) at -150°C to -196°C. All the procedures are described in more detail in the cross-referenced IND 13969.

5.1.1 Storage and Handling

PfSPZ Vaccine is cryopreserved in aliquots of 20 μL in 0.5 mL cryovials and stored in LNVP at below -150°C. The cryovials are packaged in a latched box and transported from Sanaria, Inc. to the clinical study site in a LNVP dry shipper. The LNVP dry shipper has a holding time of at least 10 days. The PfSPZ Vaccine remains in the dry shipper at the clinical study site and individual cryovials are removed from the dry shipper and thawed as needed for PfSPZ Vaccine dilution, syringe preparations and immunizations. Each cryovial is labeled indicating that it contains PfSPZ Vaccine, together with the lot number and date of manufacture. The LNVP dry shipper is labeled to indicate it is approved by International Air Transport Association (IATA) for shipment by air, conforms to UN3373 for Biological Substance, Category B, and packing instructions 650 (US 49 CFR, Part 173.199).

Transfer, receipt and maintenance of PfSPZ Vaccine from its storage site to the clinical trial site will follow SOP331 (Cryopreserved Material Transportation), provided by Sanaria, Inc. At the study site, the LNVP shipper will be continuously monitored by a data logger as well as a temperature probe according to Sanaria standard operating procedure (SOP). Receipt of the products will be documented on a tracking log by trained study staff according to Sanaria SOP.

5.1.2 Disposition and Dispensation

The clinical site must confirm that the vials of PfSPZ Vaccine have been transported and stored below -150°C according to SOP. Immediately prior to use, the cryovials will be thawed by partial submersion of the vials for 30 seconds in a 37°C ± 1°C water bath. Designated study staff will be trained by Sanaria, Inc. and then will - either alone or with Sanaria, Inc. staff - prepare, dilute (if necessary) and dispense PfSPZ Vaccine to clinical staff at the clinical study site according to SOP353 (Preparation of PfSPZ Vaccine and Diluent for Clinical Trial in Mali). The diluent is phosphate buffered saline (PBS) containing human serum albumin (HSA). PBS and 25% HSA will be provided to the clinical sites by Sanaria, Inc. for preparation of the diluent. All PfSPZ Vaccine vials, PBS and HSA that are used will be documented on inventory forms as well as documented disposition forms according to SOP. All unused PfSPZ Vaccine vials will be returned to Sanaria, Inc.

5.1.3 Administration

PfSPZ Vaccine will be injected by needle and syringe into a peripheral vein by a qualified member of the clinical team. The diluted vaccine will be prepared such that a defined volume of the suspension (not more than 0.5mL) is administered by DVI.
During administration of PfSPZ Vaccine, advanced-life-support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. The study staff administering PfSPZ Vaccine will wear gloves. The subject will stay in the clinical area for at least 30 minutes after vaccine administration.

5.2 PBS and HSA Diluent

The diluent for PfSPZ Vaccine is composed of phosphate-buffered saline (PBS) and human serum albumin (HSA). Vials of sterile PBS and HSA will be shipped to the clinical site, where diluent composed of PBS and HSA is prepared according to SOP353 (Preparation of Challenge and Diluent for Clinical Trials in Mali) provided by Sanaria, Inc.
PBS that will be used was manufactured in compliance with GMP by Sanaria, Inc. (9800 Medical Center Dr., Rockville, MD, USA). A Certificate of Analysis (CoA) is generated for each lot of PBS that is released for use in clinical studies. In addition, the PBS lots are placed on stability.

HSA is a licensed product which is approved for parenteral, IV administration to humans and is purchased by Sanaria, Inc. The HSA is repackaged at Sanaria. Repackaged HSA is released with a CoA.

5.2.1 Storage and Handling

The PBS and HSA are stored at ambient temperature within specifications (between 15°C to 30°C) that is monitored by a continuous data logger in a controlled access room. Receipt of the products will be documented on a tracking log by trained study staff according to Sanaria SOP.

5.2.2 Disposition and Dispensation

The clinical site must confirm and document that the vials of PBS and HSA have been transported and stored within specified ranges. All PBS and HSA that are used will be documented on inventory forms as well as documented disposition forms according to SOP.

5.2.3 Administration and Dosage

PBS and HSA are components of PfSPZ Vaccine diluent. The use of PBS and HSA will be according to SOP. The PfSPZ Vaccine dose will be suspended in a diluent composed of PBS and about 1% HSA in a total of 0.5 mL.

5.2.4 Accountability

PBS and HSA vial accountability will be maintained to document chain of custody from Sanaria, Inc., to study site. An inventory to account for number of vials used will be recorded and kept in the study file.

5.3 Control Product

Sterile isotonic (0.9%) normal saline will be procured in the US and shipped to Mali at ambient temperature. Like the product, normal saline is a clear liquid, making it indistinguishable from the study product when drawn up into a syringe. Normal saline will be used as a placebo, rather than a comparator vaccine being used, as currently there are no licensed vaccines available as IV formulations.

5.3.1 Storage and Handling

The normal saline is stored at room temperature in a controlled room per product standards. Each normal saline vial can either be single use or multi-use over a period of a few hours (i.e. the duration of vaccine preparation on a given day).

5.3.2 Disposition and Dispensation

The clinical site must confirm and document that the vials of normal saline have been transported and stored within specified ranges.

5.3.3 Administration and Dosage

Normal saline will be administered DVI as placebo in an equal volume to the study product.

5.3.4 Accountability

Normal saline accountability will be maintained to document chain of custody from Sanaria, Inc., to study site. An inventory to account for number of vials dispensed for each subject injection will be recorded and kept in the study file.

5.4 Antimalarial Drugs

All antimalarial medications used for the study will be maintained at the study site and administered by direct observational therapy. Coartem tablets will be purchased from commercial sources and provided by the MRTC study team to subjects. Drug accountability will be managed by the site clinical team.

5.4.1 Preparation and Administration

Coartem will be provided as tablets for oral administration. Administration is under direct observation by study staff according to dosing parameters.

5.4.2 Storage and Handling

Coartem tablets will be maintained in the manufacturer's original packaging and stored at the clinic under recommended storage conditions until prepared for dispensing.

5.4.3 Return of Study Product

Final accountability of drug supplies will be performed at the conclusion of the study. Final disposition of any remaining Coartem will be determined and documented.

5.4.4 Drugs used for Pre-emptive Treatment of Malaria

During this study, all Arms will receive pre-emptive antimalarial treatment prior to 3rd injection and 4th injection.
Artemether/lumefantrine (Coartem®) is a licensed antimalarial in the US and Mali for use for uncomplicated malaria. It has an excellent safety profile and is widely used to treat malaria. Subjects who may have any contraindications to the use of these drugs will be excluded at screening. The most common side effects reported in adults are: headache, anorexia, dizziness, asthenia, arthralgia and myalgia.

5.4.5 Drugs Used for Management of Clinical Malaria

In accordance with the Malian Government treatment guidelines, volunteers will be treated with artemether/ lumefantrine (Coartem®, 80mg/480mg per dose). Artemether/lumefantrine is a licensed antimalarial in the US and Mali for use for uncomplicated malaria. It has an excellent safety profile and is widely used to treat malaria. Subjects who may have any contraindications to the use of these drugs will be excluded at screening. The most common side effects reported in adults are: headache, anorexia, dizziness, asthenia, arthralgia and myalgia.

Clinical or symptomatic malaria for this study is defined as the presence of asexual *P. falciparum* parasites at any parasitemia level with either an axillary temperature of 37.5 °C or more or one or more of the following symptoms: headache, myalgia, arthralgia, malaise, nausea, dizziness, or abdominal pain and will be reported as an AE.

5.5 Contraindications to Vaccination

The following criteria should be checked prior to each injection and are contraindications to further injections:

- Hypersensitivity reaction following administration of the study product (PfSPZ Vaccine or placebo)
- Positive urine or serum β-hCG test prior to injection in women

Subjects will be encouraged to remain in the safety evaluation for doses already received.

5.5.1 Indications for Deferral of Vaccination

If any of the following AEs occurs at the time of the scheduled injection (vaccination with PfSPZ Vaccine or normal saline), the subject may be either vaccinated at a later date within the allowable visit window as specified in the protocol or withdrawn at the discretion of the Investigator:

- Oral temperature >37.5˚C at the time of injection will warrant deferral of injection until fever resolves (within protocol-defined window).
- Any other condition that in the opinion of the Investigator poses a threat to the individual if vaccinated or that may complicate interpretation of the safety of vaccine following vaccination.

Such individual(s) will be followed in the clinic until the symptoms resolve or the window for injection expires. No further injections will be performed if the subject does not recover (temperature ≤ 37.5˚C and/or lack of symptoms) or develops a chronic condition deemed unsafe for future injections. The subject will be monitored for safety and immunogenicity for at least 3 months after their last injection (PfSPZ vaccine or normal saline) unless the subject has withdrawn consent. If the subject does not receive any further vaccinations, then some scheduled safety blood draws may not be performed at the discretion of the PI. Blood draws scheduled to measure immune response may be obtained if possible.

5.6 Prohibited Medications

Treatment with some medications/procedures may potentially interfere with vaccine-induced immunity and/or interpretation of study endpoints. Use of any of the potentially interfering medications/procedures during the study may exclude a subject from receiving further doses of the study vaccine. However, the subject will be encouraged to remain in the study for safety evaluations. The following medications/procedures will not be permitted or may result in the withdrawal of the study subject:

- Licensed killed vaccines in the 2-week period prior to and following each vaccination or licensed live vaccines in the 4-week period prior to and following each vaccination;
- Receipt of immunoglobulins and/or any blood products up to six months prior to the first vaccination and for 30 days after administration of the last dose of vaccine;

- Chronic oral or IV administration (≥ 14 days) of immunosuppressive doses of steroids, i.e., prednisone >10 mg per day, immunosuppressants, or other immune-modifying drugs from each day of vaccination to two weeks following each vaccination;
- Any investigational drug or investigational vaccine other than the study vaccine during the study period; and
- Surgical removal of the spleen or the development of a hematologic or other disease that would interfere with normal immunity.

Medications, such as acetaminophen or ibuprofen, may be used to help relieve symptoms from vaccination and are not considered prohibited. All concomitant prescription medications, over-the-counter medications and non-prescription medications taken at the time of adverse events (all grades) or for other reasons, will be documented into the case report form (CRF).

Subjects are encouraged to contact study clinicians for any medical issues and consult with study clinicians prior to taking any medications not prescribed by the study clinician. Any medications taken by study subjects will be reviewed at every scheduled and unscheduled study visit with study staff. If a new medication has been started since the prior visit, then its potential to interact with any prescribed intervention in the study and/or interfere with the performance of the clinical trial will be assessed on a case by case basis.

If the medication is needed for the health of the study subject and is contraindicated or cautioned with protocol-defined antimalarial treatment, alternative antimalarial treatments will be made available to the study subject if clinically indicated. If the medication is needed for the health of the subject and might interfere with the vaccine by posing a safety risk to the subject, then the study subject should be withdrawn from further vaccinations and followed for safety. Use of antimalarial medications or antibiotics that have antimalarial activity administered during the study period is not exclusionary but will be documented by clinical staff and will be taken into consideration during data analysis.

When possible, study subjects should review their plans to electively initiate a new medication with study staff before starting that medication; however, medical treatment will never be withheld or delayed due to concerns regarding its effect on the execution of the clinical trial.

6 STUDY POPULATION

The study population will consist of healthy adults aged 18 – 35 years, who reside in Ouelessebougou and surrounding villages in Mali.

6.1 Clinical Site

The study will be carried out in collaboration between the Laboratory of Malaria Immunology and Vaccinology (LMIV) (National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH]), the Malaria Research and Training Center (MRTC) in Bamako, Mali, and Sanaria, Inc. MRTC is experienced in conducting Good Clinical Practice (GCP) compliant clinical trials including two malaria vaccine and drug studies.

The study will be carried out in Ouelessebougou and surrounding villages, a community located 80 km south of Bamako. Ouelessebougou village has been conducting ongoing clinical research since 2006 and is endemic for malaria with marked seasonality and a high burden of malaria in both children and adult populations.

For the purpose of vaccine trials, adequate facilities for conducting an interventional trial have been put in place at Ouelessebougou within walking distance to the residents' homes. The

study site has a research clinic with an inpatient unit. Study physicians are available 24 hours a day, seven days a week.

At Ouelessebougou, the malaria transmission is highly seasonal, with the transmission season taking place from June until December, with peak transmission in August to November. Ouelessebougou is situated in a high transmission area, with entomological inoculation rates using CDC light traps of about seven infectious bites per person over the period of August to December (Dicko et al., 2011). Malaria parasite prevalence during the transmission season varies between 20-30% in adults (unpublished data).

6.2 Recruitment

Community permission will be obtained from village elders and other community members after explanation and discussion of the study at a community meeting (see **Section 6714.2.1**). A general announcement inviting household and family members to the participating clinic to learn about the study will be made at the time of community permission, using local radio or any traditional channel of communication.

6.3 Inclusion Criteria in the main phase and booster phase

Subjects must fulfill all the following criteria to be eligible for the study:

1. Age ≥ 18 and ≤ 35 years (For booster phase, age ≥ 18 and ≤ 37 years)

2. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process

3. In good general health and without clinically significant medical history

4. Willing to have blood samples stored for future research

5. Available for the duration of the study

6. Females of childbearing potential must be willing to use reliable contraception (as defined below) from 21 days prior to Study Day 1 to 28 days after 3rd vaccination. (For the booster phase, from 21 days prior to the booster vaccination **to** 28 days post booster vaccination).

    - Reliable methods of birth control include:

        - **one** of the following: confirmed pharmacologic contraceptives (parenteral) delivery; intrauterine or implantable device. **OR**

        - **two** of the following: a documented oral or transdermal or vaginal ring contraceptives; **PLUS** condoms with spermicide or diaphragm with spermicide.

    - ***Note***- Coartem (artemether specifically) may reduce the effectiveness of systemic hormonal contraceptives, therefore additional barrier methods such as condoms must also be used during the 3 days of Coartem dosing.

- Women who are not able to get pregnant will also be required to report date of last menstrual period, history of surgical sterility (i.e. tubal ligation, hysterectomy) or premature ovarian insufficiency (POI), and will have urine or serum pregnancy test performed per protocol.

7. Enrolled in protocol # 18-I-N084 and are enrolled in the active follow up phase of the study (For the booster phase only)

8. Malaria comprehension exam completed, passed (a score of ≥80% or per investigator's discretion) and reviewed prior to enrollment

6.4 Exclusion Criteria in the main phase and booster phase

A subject will be excluded from participating in the trial if any one of the following criteria is fulfilled:

1. Pregnancy, as determined by a positive urine or serum human chorionic gonadotropin (β-hCG) test (if female)
   NOTE: Pregnancy is also a criterion for discontinuation of any further dosing or non-safety related interventions for that subject.
2. Currently breast-feeding (if female)
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the participant to understand and comply with the study protocol
4. Hemoglobin (Hb), WBC, absolute neutrophils, and platelets outside the local laboratory-defined limits of normal (subjects may be included at the investigator's discretion for 'not clinically significant' values)
5. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal (subjects may be included at the investigator's discretion for 'not clinically significant' values)
6. Infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B (HBV) (For the booster phase: re-testing NOT required for enrollment unless clinically indicated)
7. Known or documented sickle cell disease by history (Note: known sickle cell trait is NOT exclusionary)
8. Clinically significant abnormal electrocardiogram (ECG) such as abnormal QTc. (For the booster phase: re-testing NOT required for enrollment unless clinically indicated)
9. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies including urinalysis
10. History of receiving any investigational product within the past 30 days
11. Participation or planned participation in a clinical trial with an investigational product prior to completion of the follow-up visit 28 days following last vaccination OR planned participation in an investigational vaccine study until the last required protocol visit
12. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months
13. History of a severe allergic reaction(Grade 3 or higher or per PI discretion) or anaphylaxis

14. Severe asthma (defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past two years, or that has required the use of oral or parenteral corticosteroids at any time during the past two years)
15. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia
16. Known immunodeficiency syndrome (For the booster phase: re-assssment NOT required for enrollment)
17. Known asplenia or functional asplenia (For the booster phase: re-assssment NOT required for enrollment)
18. Use of:
    - Chronic (≥14 days) oral or IV corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of vaccination
    - Artemether/lumefantrine within 14 days of vaccination **unless** it has been prescribed by the study investigator as part of study procedures
    - Other antimalarials or systemic antibiotics with known antimalarial activity within 5 drug half-lives prior to the first vaccine (such as artemether, sulfadoxine-pyrimethamine, trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, ciprofloxacin, other fluoroquinolones or azithromycin)

19. Receipt of a live vaccine within the past four weeks or a killed vaccine within the past two weeks prior to Vaccination #1 and every subsequent vaccination day
20. Receipt of immunoglobulins and/or blood products within the past six months
21. Previous receipt of an investigational malaria vaccine in the last five years (For booster phase: NOT required for enrollment)
22. Known allergies or other contraindications against Coartem
23. Other condition(s) that, in the opinion of the investigator, would jeopardize the safety or rights of a participant participating in the trial, interfere with the evaluation of the study objectives, or would render the subject unable to comply with the protocol

6.5 Justification for the Exclusion of Pregnant Women

This study will not enroll pregnant and/or breastfeeding women since the effects of PfSPZ Vaccine on the developing human fetus are unknown.

Artemether/lumefantrine is considered a category C medication, there are no adequate studies in pregnant women hence only recommended for use if the potential benefit justifies the potential risk to the fetus. However, if a woman becomes pregnant after enrollment she may continue in the study for safety follow up, malaria infection evaluation and reduced vaccine immunogenicity assays as described in **Appendix A** and as outlined in **Section 15.7**.

6.6 Justification for Exclusion of Children

Children are excluded from this study because there is insufficient data on dosing or adverse events available in children at the PfSPZ Vaccine doses proposed to be used in this study.

Studies evaluating the safety, tolerability and immunogenicity of various doses of PfSPZ Vaccine are ongoing.

7 STUDY SCHEDULE

7.1 Screening

The purpose of the screening visit is to determine subject eligibility for study participation. Screening procedures include the informed consent process, Malaria Comprehension Exam, laboratory assessments (completed within 56 days of receipt of first and fourth vaccination) and clinical assessments. Screening activities can occur over multiple visits if necessary, including the day of enrollment.

In the event that a chronic illness and/or HIV, HBV, or HCV is discovered during the course of screening, long-term treatment and care will not be reimbursed by the study, but referral for continuing care can be provided to subjects.

Per national requirements for reporting communicable diseases, confirmed positive test results for HIV, HBV, and HCV will be reported to the local health department according to applicable laws and appropriate medical referrals initiated.

The following actions must be completed as part of the screening process for all subjects within the 56 days prior to first and fourth vaccination:

- Explain the study and informed consent document to the subject (For the booster phase, informed consent can be signed within 90 days prior to the 4th vaccination).
- Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.
- Ensure the subject has correctly answered ≥80% of the questions (see **Section 14.2.2**) on the Malaria Comprehension Exam.
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females, and medication use.
- Confirm that females of childbearing potential are willing to use reliable contraception from at least the period from screening through 28 days after the third vaccination in the main phase. For the booster phase, use of reliable contraception should be at least 21 days prior to vaccination up to 28 days after the fourth vaccination.
- Administer a complete physical examination, including vital signs (height, weight, blood pressure, temperature, and heart rate).
- Complete HIV pre- and post-test counseling as indicated, including follow-up contact with subject to report the results and referral for appropriate medical care if indicated.
- Obtain approximately 10 mL of blood for complete blood count (CBC) with differential and platelet count, ALT, creatinine (Cr), hepatitis B testing, hepatitis C testing, HIV testing, hemoglobinopathy testing (For the booster phase: HIV, hepatitis B, and hepatitis C testing will **only** be repeated if clinically indicated; hemoglobinopathy testing will **not** be repeated).
- Obtain urine (or serum) for pregnancy testing (for females) and urinalysis/urine dipstick for protein and blood.
- Obtain a 12-lead ECG (For booster screening: an ECG will **only** be repeated if clinically indicated).

Sickle cell testing will be completed retrospectively. History of known sickle cell disease is an exclusion at the time of enrollment. Discovery of sickle cell disease (HbSS) on subsequent laboratory testing will not result in the subject being withdrawn from the study.

Final eligibility will be determined at this point. The subject may be excluded by any of the above procedures if they meet the exclusion criteria. Acceptable ranges for hematological and biochemistry parameters defined for this study are given in **Appendix B**. (Note: parasitemia is not an exclusion criterion.)

If an abnormal finding is determined to be clinically significant, the subject will be informed, a referral letter will be issued, and the subject will be guided as to where to present for further investigation and medical care. Treatment for minor ailments may be provided by study clinicians at the study site. Decisions to exclude the subject from enrollment in the trial or to withdraw the subject from the trial will be at the discretion of the investigator.

7.2 Assignment of Groups

Enrollment **in the main phase** occurs with collection of baseline study samples on study Day -14. Randomization will occur prior to enrollment, prior to or on study Day -14. Randomization of the subjects in Arm 1 and 3a will occur first as these subjects will be enrolled first. Randomization of Arm 2 and 3b will occur together later prior to enrollment. If a subject was randomized into group 1 or 3a but was not vaccinated, they may be randomized into groups 2 and 3b if still eligible.

If a subject withdraws from the study after randomization, they can be replaced by another subject at random if withdrawal happens prior to receipt of first vaccination. Once a subject has received their first vaccination, they cannot be replaced.

For the booster phase, enrollment will occur at the receipt of the first dose of artemether/lumefantrine prior to vaccination. Enrolled subjects cannot be replaced.

During the study, the list linking randomization numbers to study product (PfSPZ Vaccine or control) will be made available only to the study statistician and associated team members, pharmacy team/syringe preparers (at the start of the study), independent safety monitor (if needed to review as outlined in **Section 11.15.2**), and DSMB chair (if needed for closed session unblinded review). On vaccination days, the vaccines associated with each randomization number will be obtained from the pharmacist.

To ensure proper identification of study subjects, following subject enrollment all subjects will receive an identification card with their photo on it to present at the clinic with each study visit.

7.3 Detailed Study Procedures

Detailed study procedures are outlined in **Appendix A** of the protocol.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Photographs of Rash or Injection Site Reactions

If a subject develops a rash or injection site reaction, photographs may be taken by the investigators. These photographs will not include the subject's face or any identifying scars, marks, or tattoos.

8.2 Clinical Laboratory Testing

Using standard techniques, the clinical laboratory will perform the following tests:

1. Complete blood count (CBC) plus white blood cell (WBC) differential and platelet count
   - The following CBC parameters will be assessed for safety throughout the trial: WBC, absolute neutrophil count (ANC)/absolute granulocyte count (AGC), hemoglobin (Hb), and platelet count.
2. Serum creatinine (Cr)
3. Alanine aminotransferase (ALT)
4. HBsAg test (can include rapid diagnostics, ELISA, PCR if indicated) (at screening only in the main phase. For the booster phase, ONLY if clinically indicated)
5. HCV test (can include rapid diagnostics, ELISA, PCR if indicated) (at screening only in the main phase. For the booster phase, ONLY if clinically indicated)
6. HIV test (can include rapid diagnostics, ELISA, Western Blot if indicated) (at screening only in the main phase. For the booster phase, ONLY if clinically indicated)
7. Urine dipstick and/or Urinalysis (at screening for the main and booster phases only)
8. Urine and/or serum pregnancy testing (β-hCG)

Additional clinical testing includes the following but will be completed retrospectively by other laboratories as noted below:

1. Hemoglobinopathy testing - testing for normal adult hemoglobin (HbAA), hemoglobin c trait (HbCC), hemoglobin sickle cell trait (HbAS), hemoglobin sickle cell disease (HbSS)

8.3 Electrocardiogram

8.3.1 Electrocardiogram for the Study

Electrocardiograms (12-lead ECGs) will be performed during screening in the main phase and as needed throughout the study and will be read by the study site physician in Mali, a Mali cardiologist or NIH study cardiologist or their representative. Subjects with QT interval (QTc) > 460 ms may be excluded as Coartem may prolong QTc. Subjects with clinically significant abnormalities will also be excluded from the study.

8.4 Malaria Diagnostics

8.4.1 Malaria Blood Smears

The gold standard for malaria diagnosis and evaluation of VE endpoints is the detection of malaria parasites on Giemsa-stained thick blood films. Blood films will be prepared at the specified time points or when clinically indicated and will be examined by technicians with a documented experience in slide reading following the SOP established for malaria slide reading

within the MRTC (Guindo et al., 2012), and in consideration of the modifications suggested for CHMI trials (Laurens et al., 2012). Slides are considered positive if at least two unambiguous *P.falciparum* parasites per slide are identified and confirmed by a second microscopist. Positive results will be reported promptly to the study Principal Investigator or designee.

Thick blood smears will be prepared from the blood remaining in the IV cannula, or (at time points when no IV blood collection is planned) from a finger prick sample. The smears will be examined microscopically.

Thick blood smears will be used for diagnosis throughout the study.

8.4.2 Malaria qPCR

While detection of parasites on thick blood smears remains the most common primary endpoint in human challenge trials, both PCR- and nucleic acid sequence-based amplification (NASBA)-based methods have been increasingly used to support blood smear data in malaria vaccine trials (M. Walther et al., 2005; M. Walther et al., 2006). These research molecular assays have significantly increased sensitivity for detection of *P. falciparum* blood-stage infection approaching 20 parasites/mL, often resulting in diagnosis 2-4 days earlier than by paired thick blood smears (Hermsen et al., 2001; Schneider et al., 2004; M. Walther et al., 2005). Quantification of parasite density by these methods allows evaluation of parasite growth curves for assessing the utility of partially-effective vaccine candidates. LMIV has also developed a research qPCR that detects 18s of *P. falciparum* with a detection limit of at least 20 parasites/mL that will be used during the study for comparison to traditional thick blood smears.
P. falciparum qPCR may be performed from all scheduled visits with a malaria blood smear noted (see **Appendix A**) to capture infections that remain below the detection limit for microscopy. For subject convenience, a finger prick sample can be used for both preparation of the microscopy slide and for DNA preservation.

8.5 Unscheduled Blood Smear Positive Visits

Each time a subjects has detected *P falciparum* malaria parasites on thick blood smear (whether or not they were symptomatic; scheduled or unscheduled visits), he/she will be asked to the clinic to provide an additional blood sample within 24 hours of the clinic visit (the next calendar day) for the following as seen in **Appendix A**:

- 0.5 mL ethylene diamine tetraacetic acid (EDTA) microtainer: for whole blood ex-vivo assays if sample not obtained within the last two days of the positive blood smear .(For the booster phase, only collect if the sample has not been obtained within the last 7 days).
- 4 mL EDTA tube: to obtain RNA and DNA for the study of host and parasite transcriptome (RNA) and parasite genotype (DNA) and to obtain plasma for proteomics studies if sample not obtained within the last 28 days of the positive blood smear
- 0.5 mL for qPCR if sample not obtained within the last two days of the positive blood smear (For the booster phase, NOT required)

Note: if a blood smear is positive for *P.falciparum*, documentation in a smear positive CRF is required whether these additional samples were collected or not.

8.6 Immunologic Laboratories

As indicated in the objectives, assays will be conducted to assess immunogenicity in addition to safety as described above. Laboratory assays to assess immune response to PfSPZ Vaccine will be performed at the LMIV, NIAID, NIH, Sanaria Inc, and NIH Center for Human Immunology according to standard laboratory procedures.
These assays include:

1. Binding ELISA for antibodies to *P. falciparum* liver stage and blood stage antigens (which includes but not limited to: CSP, sporozoite surface protein 2, liver stage antigen 1, erythrocyte binding antigen 175, merozoite surface protein 1, merozoite surface protein 5, and exported protein 1 [PfCSP, PfLSA-1, PfEBA-175, PfMSP-1, PfMSP-5, malaria protein EXP-1]
2. (IFN-γ) ELISPOT assay and multi-parameter flow cytometry with intracellular cytokine staining on peripheral blood mononuclear cells in *P. falciparum* liver- stage antigens (CSP, LSA-1) and PfSPZ
3. B and T cells studies to analyze immunologic responses

Sanaria, Inc. will also assess antibodies to whole PfSPZ by immunofluorescence assay (IFA) and inhibition of sporozoite invasion assay (ISI) and to asexual erythrocytic stage parasites by IFA as described (Epstein et al., 2011; Seder et al., 2013) and also by protein microarrays (Antigen Discovery Inc).

In addition, using plasma, sera, peripheral blood mononuclear cells, or their component parts (e.g., purified IgG), Naval Medical Research Center will perform assays of humoral and cellular immunity that may be associated with protective activity.

Laboratory assays to assess immune responses to novel pre-erythrocytic antigens will be performed in the PEVA Consortium laboratories at the LMIV, NIAID, and NIH according to standard laboratory procedures. The target proteins are novel antigens that confer protection against liver stage malaria in rodent malaria models (*P. yoelii*, *P. berghei*) according to vaccination studies conducted by Seattle BioMed and LMIV. The novel antigens to be used for these laboratory assays include PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113, using the identifiers in the PlasmoDB database (www.plasmodb.org). These antigens were initially selected on the basis of their gene expression during early liver stage development of *P. falciparum*, and preliminary testing shows that these antigens are immunologically recognized by individuals previously exposed to *P. falciparum*. The potential utility of these antigens as pre-erythrocytic vaccines has been supported by animal studies, wherein orthologues of these genes incorporated in DNA vaccines induce protective immunity in mice that significantly reduces the liver stage development of *P. berghei* and *P. yoelii* parasites. The assays included in this study can confirm that individuals receiving CVac with PYR develop immune responses to pre-erythrocytic antigens and can provide additional data by which to assess the potential for these antigens to be developed as subunit vaccines to prevent infection. The long-term objective of the PEVA consortium is to identify antigens that individually, or in combination with CSP or other antigens, will induce a high level of pre-erythrocytic immunity that is protective against *P. falciparum*.

The assays to be performed include:

1. Binding ELISA for antibodies to *P. falciparum* pre-erythrocytic antigens (PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113)

2. IFN-γ ELISPOT/Intracellular cytokine staining (ICS) assay on peripheral blood mononuclear cells in *P. falciparum* pre-erythrocytic antigens (PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113)

8.7 Transcriptomic analysis

Whole genome transcriptional profiling will be performed to explore possible gene expression profiles or pathways that predict optimal responses to vaccination. Gene expression profiling following vaccination will allow the predictive capacity of eventual protected and unprotected vaccinees, and thus will assist in defining the correlates of protection induced by vaccination. Transcriptional analyses will be performed on whole blood collected as outlined in **Appendix A**. Blood will be collected via venous puncture and placed in PAXGene tubes to preserve RNA integrity until the RNA is extracted. The molecular profiling encompasses the identification of RNA transcripts present in all humans, which are induced or repressed after each vaccination. This does not represent genetic testing of individuals or their DNA.

9 RESEARCH USE OF STORED HUMAN SPECIMENS

Intended Use: Samples and data collected under this protocol will be used to study malaria and related diseases and possible adverse reactions to vaccination.

Storage: Access to stored research samples will be limited using either a locked room or a locked freezer. Temporary storage of samples collected in Mali, prior to shipment to LMIV, may occur at the Core Immunology Laboratory or the MRTC CAP laboratory. Samples will be stored at the LMIV in Rockville, MD or at LMIV's designated repository, Thermo Scientific, Rockville, MD, with the exception of retention specimens which may be kept at the MRTC in Mali for quality control. Samples and data will be stored using codes assigned by the investigators or their designees. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples will be tracked using a sample-tracking software program, e.g., Freezerworks.

Disposition at the Completion of the Protocol: In the future, other investigators (both at the NIH and outside) may wish to study these samples and/or data. In that case, Institutional Review Board (IRB) approval must be sought prior to any sharing of samples. Any clinical information shared about the sample with or without patient identifiers would similarly require prior IRB approval.

At the completion of the protocol (termination), samples and data will either be destroyed or, after IRB approval, transferred to another existing protocol.

Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB: Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of Protocol

Deviation and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIAID IRB.

Consent to allow long term storage of study samples is a part of the inclusion criteria for this study. However, if a subject decides following enrollment not to have their samples stored, the PI or designee will destroy all known remaining samples and report this destruction to the subject and the NIAID IRB and Faculty of Medicine, Pharmacy and Odonto-Stomatology Ethics Committee (FMPOS EC). This decision will not affect the subject's continued participation in this protocol or any other protocols supported by the NIH.

10 DATA SHARING PLAN

In NIH's view, all data should be considered for data sharing. Data should be made as widely and freely available as possible while safeguarding the privacy of subjects, and protecting confidential and proprietary data. We recognize that the public dissemination of our scientific results can facilitate the creation of collaborative efforts with domestic and international collaborators. Furthermore, we recognize that the proposed project may result in novel ideas for new methods, technologies, and data that could benefit the entire research community. Therefore, final research data will be shared openly and timely in accordance with the most recent NIH guidelines (http://grants.nih.gov/grants/policy/data_sharing/) while being mindful that the confidentiality and privacy of participants in research must be protected at all times. Timelines for distribution of data will vary depending on any required restrictions in accordance with federal and/or institutional policies and guidelines. In general, we expect de-identified data will be available through NIH-funded or approved public repository, speaking engagements and publications, presentations at scientific symposia and seminars. Effort will be made to publish our research findings in scientific journals. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. For tools, reagents, data and model organisms generated by the proposed study, pending third parties' rights, LMIV will transfer materials to outside researchers in both the private and public sectors under a Material Transfer Agreement or Research Collaboration Agreement.

11 ASSESSMENT OF SAFETY

11.1 Documenting, Recording, and Reporting Adverse Events

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject's medical record/source document,
- recorded on the Adverse Event Case Report Form (AE CRF) or electronic database, and
- reported as outlined below (e.g., IND sponsor, IRB, FDA).

A study clinician will be available during the study period and will be available to the study subjects at all times. Should a subject call a study clinician to report an AE, it will be discussed with the PI and documented, recorded, and reported appropriately.

11.2 Definitions

Adverse Event (AE): An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR): An AR is an adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR): A SAR is an adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse

event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE): A SAE is defined as an AE that results in any of the following outcomes:

- death
- life threatening (i.e., an immediate threat to life)
- inpatient hospitalization or prolongation of an existing hospitalization
- persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- congenital anomaly/birth defect
- medically important event

Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or result in hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event: An AE is considered unexpected if it is not listed in the Investigator's Brochure (IB) or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is a SAR that is both serious and unexpected.

Unanticipated Problem (UP): An UP is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document, IB, or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event (UPnonAE): An UPnonAE is an UP that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

New Onset of Chronic Illness (NOCI): A NOCI is defined as a diagnosis of a new medical condition that is chronic in nature, including those potentially controllable by medication (e.g., diabetes, asthma). Any NOCI will be recorded in the same manner as unsolicited AEs.

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as major or minor.

Major Deviations - Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.

Minor Deviations - Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Noncompliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research or the requirements or determinations of the IRB, whether intentional or not

11.3 Investigator Assessment of Adverse Events

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.
All solicited (see **Table 6** below) and unsolicited AEs will be recorded through Day 7 after each injection. Injection site reactions will be assessed until Day 7 after injection (PfSPZ Vaccine or normal saline) or until resolved.

After the periods specified above only unsolicited AEs, SAEs, UPs, and NOCIs will be recorded.

Table 6: Solicited Adverse Events

Laboratory adverse events[1]	
Hemoglobin (decreased hemoglobin)	Platelet count (thrombocytopenia or thrombocytosis)
WBC (leukopenia or leukocytosis)	Creatinine (Cr) (increased Cr)
ANC (neutrophil count decreased) or AGC (granulocyte count decreased)	ALT (increased ALT)
Local reactogenicity (secondary to PfSPZ Vaccine/normal saline) – through Day 7 post injection	
Injection site pain/tenderness	Injection site induration
Injection site erythema/redness	Injection site swelling/edema
Injection site pruritus	Injection site bruising
Systemic reactogenicity (secondary to PfSPZ Vaccine/normal saline) – through Day 7 post injection	
Rash	Urticaria
Generalized pruritus	Generalized Edema
Headache	Fever or feverish
Chills	Malaise/Fatigue
Myalgia	Arthralgia
Sweats	Diarrhea
Back Pain	Chest Pain (non-musculoskeletal)
Nausea/Vomiting	Abdominal Pain

[1] Note absolute lymphocyte counts will be capture on CRFs for use for research assessments. If clinically significant changes as determined by PI, these may also be reported as AEs as noted below.

Any laboratory abnormalities (other than those specified as safety labs in the protocol as defined by the values in the toxicity table) should be reported as AEs if they require intervention. Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments, or concomitant treatment. In addition, any medically important laboratory abnormality may be reported as an adverse event at the discretion of the investigator. This could include a laboratory result for which there is no intervention, but the abnormal value suggests a disease or organ toxicity. In addition, similar to solicited AEs, all laboratory AEs as defined in **Appendix C**, will be collected and graded for severity through 7 days after each vaccination until resolved.
The Investigator will assess all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following sections in the protocol.

11.3.1 **Severity**

Severity of AEs will be assessed by the investigator as described in **Appendix B.** AEs not included in the Appendices will be graded for severity using the followings definitions as seen in **Table 7.**

Table 7: AE Severity Grading Definitions

Severity	Definition
Grade 1 (Mild)	No interference with activity, may use one dose of an over the counter medication
Grade 2 (Moderate)	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity
Grade 3 (Severe)	Activities of daily living limited to <50% of baseline, medical evaluation/therapy required
Grade 4 (Life-Threatening)	Extreme limitation in activity, significant assistance required; immediate medical intervention or therapy required to prevent death
Grade 5	Death

11.3.2 **Causality**

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship
  OR
- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship
  OR
- definitely due to an alternative etiology

Note: Other factors will also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

The degree of certainty with which an AE can be attributed to administration of the study vaccine will be determined by how well the event can be understood in terms of one or more of the following:

- The event being temporally related with vaccination or reproduced on re-vaccination;
- A reaction of similar nature having previously been observed with this type of vaccine and/or formulation;
- The event having been reported in the literature for similar types of vaccines; and/or
- Whether or not there is another identifiable cause.

All local (injection site) reactions will be considered causally related to vaccination. All malaria cases will be reported as not related to vaccination unless results indicate otherwise. Asymptomatic parasitemia (positive blood smears without related malaria clinical symptoms) will not be reported as an AE. Clinical malaria will be reported as an AE.
Reports will further classify AEs as follows:

- Related - all AEs that are assessed as definitely, probably, or possibly related.
- Unrelated - all AEs assessed as unlikely or definitely not related.

When reporting to regulatory authorities and IRBs is needed, AE relationship will be determined as noted above.

11.4 Investigator Reporting Responsibilities to the Sponsor

11.4.1 Adverse Events

Line listings, frequency tables and other summary AE data will be submitted to the IND sponsors when needed for periodic safety reviews, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

11.4.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) will be reported and sent to the IND sponsor by fax (SAE fax line: 240-306-0596) or email attachment. Deaths,

immediately life threatening and all possibly, probably or definitely related SAEs will be communicated by telephone, fax, email or automated report via the data management system by the PI **within 24 hours** of site awareness of occurrence to the IND sponsor. All other SAEs will be reported to the IND sponsor **within three business days** after the site becomes aware of the event. The PI must document that the communication is received and acknowledged.

Sanaria, Inc.
SAE Fax: 240-306-0596

Individuals:
1. Stephen L. Hoffman, M.D.
Tel: 240-403-2701(office)
Tel: 240-299 3178 (mobile)
Email: slhoffman@sanaria.com

2. Thomas L Richie, M.D., Ph.D.
Tel: 240-403-2727 (office)
Tel: 301-466-7943 (mobile)
Email: trichie@sanaria.com

3. Tooba Murshedkar, MS
SAE Fax: 240-306-0596
Email: tmurshedkar@sanaria.com

In Mali – the clinical site investigator will also notify the LMIV PI and the site medical monitor in Mali by email, fax, or telephone within one working day of notification of an SAE occurrence.

LMIV Contact Information:
Patrick Duffy, MD
Office 301 761 5089
Fax 301 480 1962
Email: Patrick.Duffy@nih.gov

Independent Safety Monitor:
Mamadou Dembele, MD
Service Medicine Interne
Centre Hospitalo-Universitaire du Point G
+ 223 2022 5003 or mobile: +223 7604 93 87
Email: hassiramadydembele@yahoo.fr

11.4.3 **Unanticipated Problems (UPs)**

All UPs that are also adverse events will be reported to the IND sponsor on the NIH Problem Report Form sent by fax or email attachment no later than 7 calendar days of site awareness of the event.
UPs that are not AEs will also be reported to the IND sponsor.

11.4.4 **Pregnancy**

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the IND sponsor and LMIV via fax or email within three business days from the site awareness of the pregnancy. Pregnancy outcome data (e.g., delivery outcome, spontaneous, or elective termination of the pregnancy) will be reported to the IND sponsor and LMIV within three business days of the site's awareness of the outcome on a protocol-specified form.
In the event of pregnancy, the following steps will be taken:

- Discontinue the study agent
- Unblind per the site unblinding procedures if the pregnancy occurs within 4 weeks after the last vaccination.
  Note: If pregnancy occurs **more than 4 weeks after** the last vaccination, the subject should **ONLY** be unblinded if she meets the criteria for intentional, unscheduled unblinding in **Section 11.4**.
- The subject will be withdrawn from receiving any further investigational products but will continue in follow-up for safety, malaria infection evaluation and modified immunogenicity assays as outlined in **Appendix A**.
- Report to FMPOS EC as an informational item
- Report to NIAID IRB as an informational item
- Report to DSMB, Sponsor Medical Monitor, and Site Medical Monitor
- Advise research subject to notify the obstetrician of participation in the study

11.5 **Reporting Procedures to NIAID IRB and FMPOS EC**

11.5.1 **Reporting to the NIAID IRB**

UPs, non-compliance and other reportable events will be reported to the NIH IRB as per Policy 801.

11.5.2 **Reporting to the FMPOS EC**

Events reported to the NIAID IRB will simultaneously be reported the the FMPOS EC. In addition, SAEs, related or not related to the research will be reported to the FMPOS EC within 72 hours of investigator awareness, regardless of expectedness.

11.6 **Additional Investigator Reporting Responsibilities to the Local IRB (NIAID IRB and FMPOS EC)**

Investigators are responsible for submitting any IND FDA Safety Reports and UP summaries that are received from the IND sponsor to their local IRB/EC. Investigators must also comply with all local IRB/EC reporting requirements.

11.7 **Reporting to the Data and Safety Monitoring Board**

As agreed with the Office of Clinical Research Policy and Regulatory Operations (OCRPRO), a DSMB chartered by the IND sponsor, Sanaria, Inc. will be used for this study instead of the NIAID Intramural DSMB.

The DSMB will review the study prior to initiation, after completion of the vaccination series and at study close (as outlined in **Figure 5**). Prior to the administration of the booster

vaccine, the DSMB will review the data from the main phase and the plans for the booster phase. The board may convene additional reviews as necessary, and will issue recommendations concerning continuation, modification, or termination of the study. All SAEs will be reported by the PI to the sponsor immediately upon becoming aware of them. All SAEs that are possibly, probably or definitely related to the study agent, UPs, and safety reports (as available) will be reported by the sponsor to the DSMB.

The DSMB will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. The sponsor will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The sponsor will notify the DSMB at the time pausing criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

11.8 Follow-up Adverse Events and Serious Adverse Events

AEs that occur following receipt of a single vaccination are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period will be followed until the final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF (if the CRF is still open) and the SERF.

SAEs that occur after the study follow-up period (six months following the last vaccination) that are reported to and assessed by the Investigator to be possibly, probably, or definitely related must be reported to the study IND sponsor as described above.

11.9 Sponsor's Reporting Responsibilities

SUSARs as defined in 21 CFR 312.32 and determined by the IND sponsor will be reported to FDA and all participating Investigators of related Sanaria trials as IND Safety Reports.
The IND sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

11.10 Pausing Criteria for the Study

The PI will closely monitor blinded study data as they become available and will make determinations regarding the presence and grading of AEs. The AEs will be evaluated with regard to the known complications associated with administration of vaccine components. If a dose of vaccine is considered unacceptably reactogenic (as described in the following criteria), the study will be paused. No new enrollments and no further vaccinations will be administered by the investigators until reviewed by the DSMB and study IND sponsor. A report of DSMB recommendations will be submitted to the IRBs. The following criteria will be used to define unacceptable reactogenicity of the malaria vaccine (AEs that are possibly, probably, or definitely related to the vaccine will be considered "Related" and will be summarized as such):

1. One or more subjects experience an SAE as defined in **Section 11.4.2** of this protocol that is determined to be possibly, probably, or definitely related to the vaccine or placebo or
2. One or more subjects experience a hypersensitivity reaction (e.g. anaphylaxis, diffuse urticaria) that is probably or definitely related to the vaccine or placebo; or
3. Any severe clinical illness occurs that is not explained by a diagnosis that is unrelated to injection; or

4. Thirty percent of subjects in any dose arm experience the same Grade 2 or higher laboratory abnormality (see **Appendix B**), or Grade 3 systemic AE that is determined to be possibly, probably, or definitely related to the injection (PfSPZ Vaccine or placebo) as defined in this protocol or
5. Ten percent of subjects in any arm experience Grade 3 or higher local reactogenicity; or
6. Any safety issue that the study PI or IND sponsor determines should pause the study.

The IRBs, the NIAID, the FDA, or other government agencies may discontinue the study at any time. Subsequent review of serious, unexpected, and related AEs by the DSMB or IRB, the IND sponsor, the FDA, and other regulatory authorities may also result in suspension of further administration of vaccine at the clinical site (or, in the case of the DSMB, a recommendation to the Sponsor that the study should be suspended). The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and administration of vaccine for the entire study as applicable.

11.10.1 Reporting of Study Pausing

If a pausing requirement is met, a description of the event(s) or safety issue will be reported by the PI or Site Investigator within one business day to the IND sponsor by fax or email.
The site investigator will inform the LMIV PI, the ISM, and the local IRB that a pausing rule has been met according to their requirements. The LMIV PI will notify the NIAID IRB. The IND sponsor will notify the DSMB as well as all sites conducting Sanaria-sponsored studies, or studies using Sanaria related products that the study has been paused.

11.10.2 Resumption of a Paused Study

The IND sponsor, in collaboration with the PI and DSMB will determine if it is safe to resume the study. The IND sponsor will notify the site investigators of this decision. The conditions for resumption of the study will be defined in this notification. The site investigator will notify their local IRB(s) of the study pause and of the decision to resume the study.

11.11 Pausing Criteria for a Subject

The decision to suspend administration of the study agent(s) for a single subject or for all subjects in a specific group requires discontinuation of use of the study agent to any study subject(s) until a decision is made whether or not to continue study agent in the study.
The pausing criteria for a single subject or for the subjects in this study include:

- A subject experiences an SAE or ≥ 2 or more Grade 3 or greater AE (excluding laboratory assays) that is unexpected (as determined by the IND sponsor) and is possibly, probably, or definitely related to the study agent;
  OR
- Any safety issue that the Site Investigator determines should pause administration of the study agent to a single subject.

The IND sponsor, in collaboration with the PI, may also pause for an individual subject if a safety concern is identified during routine aggregate data analysis.

11.11.1 Reporting of Pausing for a Subject

If a pausing requirement is met, a description of the AE(s) or safety issue must be reported by the site Investigator by fax or email within one business day to the IND sponsor and LMIV PI. The

PIs will notify the ISM, and the local IRB (NIAID IRB, FMPOS EC). The IND sponsor will report this to the DSMB.

11.11.2 Resumption of a Paused Subject

The IND sponsor in collaboration with the PI and the DSMB will determine if it is safe to resume administration of the study agent to the subject. The IND sponsor will notify the Site investigators of this decision. The site investigators will notify their local IRB(s) of the decision to resume administration of the study agent prior to resumption.

11.12 Discontinuation of Study

Sanaria, Inc. as the study sponsor, the NIAID IRB, FMPOS EC, and the FDA may terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of an AE in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory
- Data recording is inaccurate or incomplete
- Investigators do not adhere to the protocol or applicable regulatory guidelines in conducting the study

11.13 Withdrawal of an Individual Subject

A subject will not be considered to have completed the trial if any of the following reasons apply:

1. *Research terminated by Sponsor or Investigator* – applies to the situation where the entire study is terminated by the sponsor or investigator, or other regulatory authority for any reason.
2. *Withdrawal of consent* – applies to a subject who withdraws consent to participate in the study for any reason.
3. *Noncompliant with protocol* – applies to a subject who does not comply with protocol-specific visits or evaluations, on a consistent basis, such that adequate follow-up is not possible, and the subject's safety would be compromised by continuing in the trial. This also applies to a subject who is lost to follow-up and is not reachable by telephone or other means of communication and cannot be located.
4. *Completed follow up after developing an AE* – applies to a subject who is withdrawn from study due to an AE, serious or otherwise. Any grade 3 or greater AE that is assessed as possibly, probably, or definitely related to vaccination, PfSPZ Vaccine or placebo (other than local reactions lasting <72 hours, or systemic reactions lasting <48 hours) will result in withdrawal of the subject from further vaccinations. Subjects may also be withdrawn for any AE that would cause continued participation in the study to not be in the best interest of the subject, as per the investigator's judgment. Any subject who will notreceive any further vaccination because of an AE related to study agent will be followed for safety until at least resolution of that AE and will be encouraged to remain in the safety evaluation for the duration of the study.
5. *Other* – is used when previous categories do not apply and a written explanation is required.

If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision will be recorded in the source documents and CRFs. Any subject who has received at least one dose of vaccine will be encouraged to remain in the safety evaluation for the duration of the study. The subject's data will be included in the safety and immunogenicity analysis. If a subject fails to complete all planned vaccinations because of an AE or SAE, the subject will be followed until resolution or stabilization of the event. If a subject withdraws, the investigator will make a reasonable effort to determine the reason.

11.13.1 Replacement of Withdrawn Subjects

Subjects who have received at least one vaccination and who withdraw or are terminated from the study prior to completion will not be replaced. In the main phase, subjects withdrawn before the first vaccination may be replaced.

11.14 Unblinding for the Study

Intentional, unscheduled unblinding may occur if a subject experiences a SAE that the treating clinician and/or site PI believes warrants unblinding to provide appropriate clinical management of the subject. The request for unblinding may be requested by the site PI or designee, sponsor, sponsor medical monitor, independent medical monitor, and DSMB. If non-emergent, all parties should be notified to discuss prior to unblinding taking place. If unblinding is requested, an "Unblinding Report" should be documented per standard operating procedures and submitted to the study statistician for formal unblinding.

If emergency unblinding is required, the PI or designee will contact the study statistician or study pharmacist for unblinding. The sponsor will be informed within one business day that the unblinding was necessary and a submitted "Unblinding Report" will be provided within 2 business days.

Subjects who are unblinded will be encouraged to remain in the study to be followed for safety.

For subjects who do not enroll in the booster phase, scheduled unblinding of the subject by the unblinded pharmacist will occur on Study Day 295 (*Arms 1 and 3a*) and Study Day 211 (*Arms 2 and 3b*) at the completion of the subject's final visit as already outlined in **Appendix A** per site procedures. For subjects enrolled in the booster phase, scheduled unblinding by the unblinded pharmacist will occur on Study Day 561 (*Arms 1 and 3a*) and Study Day 477 (*Arms 2 and 3b*). All primary and secondary endpoints will have been completed at this time. Individuals directly continuing AE assessment, including study investigators, or performing assays for exploratory endpoints will remain blinded to individual randomization until assessment or assays are completed.

11.15 Safety Oversight

11.15.1 Sponsor Medical Monitor

A medical monitor, representing the IND sponsor (Sanaria, Inc) has been appointed for oversight of safety in this clinical study. The sponsor medical monitor will be responsible for performing safety assessments.

11.15.2 Independent Safety Monitor (ISM) in Mali

An independent safety monitor (ISM) in Mali will review the study prior to initiation and will be available to advise the investigators on study-related medical issues and to act as a representative

for the welfare of the subjects. The ISM will conduct independent safety monitoring and recommend appropriate action regarding adverse events and other safety issues. The ISM is an expert in the field of oversight of clinical trials conducted in Mali and internal medicine, specifically in the population under study in Mali. The ISM does not have direct involvement in the conduct of the study and does not have other interests with any collaborating pharmaceutical firms or their competitors.

All serious adverse events, all UPs, and all FDA IND Safety Reports will be reported by the PI to the ISM prior to or at the same time they are submitted to the IRB or IND sponsor. The ISM will be notified immediately if any pausing rule is met and the ISM will provide recommendation for continuation, modification, or termination of the study. The PI will also notify the medical monitor if intentional or unintentional unblinding occurs.

11.15.3 Data and Safety Monitoring Board (DSMB)

As agreed with OCRPRO, for this study a DSMB chartered by the IND sponsor, Sanaria, Inc. will be used instead of the NIAID Intramural DSMB.

The DSMB will review the study prior to initiation and as outlined in **Figure 5** and **Section 11.7**. The board may convene additional reviews as necessary, and will issue recommendations concerning continuation, modification, or termination of the study. All SAEs, UPs, and all IND Safety Reports will be reported by the sponsor to the DSMB.

The DSMB will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. The sponsor will notify the DSMB at the time pausing criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit written DSMB summary reports with recommendations to the IRB(s).

12 CLINICAL MONITORING

12.1 Site Monitoring Plan

As per International Conference on Harmonisation (ICH)-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements: for the Office for Human Research Protections (OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms) and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA,

the IND Sponsor (Sanaria), the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

13 STATISTICAL CONSIDERATIONS

13.1 Endpoints and Statistical Methods

13.1.1 Safety analysis

Safety will be assessed by line listing and tables at the individual level. Intention-to-treat (ITT) group comparisons, between the vaccine arms and the control arm, will be performed in terms of the proportions of solicited AE, related AE and SAE. Other concerning trends will be investigated as needed or requested by the PI, ISM, IND sponsor, and/or DSMB.

13.1.2 Vaccine efficacy analysis

Primary efficacy analysis will be based on time to infection, which is defined as the time to the first positive blood smear. Arms will be compared by logrank test for interval-censored data (using the interval package in R). VE will be estimated assuming a Weibull model and allowing for interval censoring and stratification by village (survival package in R).

The proportions of infection will be also compared across arms by the exact binomial test, or the Exact Cochran-Mantel-Haenszel test stratifying on village if needed (using the mantelhaen.test in R with the exact option set to true, this is a conditional exact test). Subjects that are censored prior to the last day of planned follow up will be removed from the analysis and censoring will be assumed to be completely at random. A sensitivity analysis may be performed where all censored subjects are treated as either cases or non-cases in either arm.

Under either of these analyses, we will perform an ITT and a per-protocol analysis. Under the per-protocol analysis, subjects that drop out prior to receiving the full number of vaccinations will not be included in the analysis.

The same efficacy analyses will be performed at the end of the main phase and the end of the booster phase.

13.1.3 Immunogenicity analysis

We will characterize and compare immunogenicity responses between vaccine and placebo arms by Wilcoxon rank sum test or two-sample t-test after proper transformation. We will also compare the immunogenicity response before and after vaccination by Wilcoxon signed rank test or paired t-test after proper transformation. Figures of immune responses in each study arm will be provided to depict the pattern of change in immune responses.

We will explore the impact of PfSPZ Vaccine on the genotype and transcriptome profile of parasites isolated from study subjects using a linear regression or a generalized linear regression.

The same immunogenicity analysis will be carried out at the end of the main phase and the end of the booster phase.

13.1.4 Interim analysis

An interim analysis on efficacy will be performed after the first peak transmission season, approximately in December 2018. The same efficacy analysis methods will be applied to the interim analysis in both ITT and per protocol cohorts. The interim analysis will be performed to assist regimen selection in future studies. The analysis results will not affect the final analysis of the study. The interim analysis will completed by the study statistician. Analysis will be reviewed by LMIV senior investigator and PI, MRTC senior investigator, the sponsor and DSMB. Subjects, site clinicians and site personnel conducting clinical assessments and LMIV

and MRTC laboratory personnel conducting study related assays will remain blinded until after scheduled unblinding at the completion of the study.

13.2 Sample Size Consideration

Sample size consideration is primarily based on VE evaluation. Seventy subjects will be enrolled in each arm. If the annual drop-out rate is between 15% and 28%, there will be 50 to 60 available subjects for efficacy evaluation at one year after study initiation. With 50 samples per arm, the study is capable of detecting the protective efficacy of vaccine with 88% power if VE is 45% and the background infection rate is 0.7 (see **Table 8**). With 60 samples per arm, the study has 83% power if VE is 45% and background infection rate is 0.6. For safety evaluation, with 70 subjects in each arm, there is 90% probability of observing at least one AE in the arm if the adverse event rate is no less than 0.033.

Based on data from existing studies, the number of participants in the booster phase will be close to that in the main phase. The above sample size consideration should approximately apply to the booster phase.

Table 8: Power for group-wise comparison based on logrank test on time to infection

Sample Size per Arm	Background Infection Rate	Vaccine Efficacy (VE)	Power
50	0.6	0.45	76
50	0.6	0.3	44
50	0.6	0.25	33
50	**0.7**	**0.45**	**88**
50	0.7	0.3	59
50	0.7	0.25	45
60	**0.6**	**0.45**	**83**
60	0.6	0.3	51
60	0.6	0.25	39
60	0.7	0.45	93
60	0.7	0.3	67
60	0.7	0.25	52
70	0.6	0.45	89
70	0.6	0.3	57
70	0.6	0.25	44
70	0.7	0.45	96
70	0.7	0.3	73
70	0.7	0.25	59

13.3 Randomization and Blinding

Participants in arms 1 and 3a will be enrolled and randomized before the enrollment and randomization of participants in arms 2 and 3b. The study will be randomized with block randomization and stratified by village. To accomplish this, a list of all enrolled subjects will be given to the study statistician at least 2 days prior to the desired time for the first randomization. To prevent dropout before first vaccination, randomization and first administration of vaccination or placebo should occur as close in time as possible.

14 HUMAN SUBJECT PROTECTIONS AND ETHICAL OBLIGATIONS

This research will be conducted in compliance with the protocol, GCPs, and all applicable regulatory requirements.

14.1 Institutional Review Board

A copy of the protocol, informed consent forms (ICFs), and other study related information to be completed by subjects, such as questionnaires, diary cards, medical history forms, and any proposed advertising/recruitment materials or letters to the subjects will be submitted to the reviewing IRBs for written approval. The investigator must submit and obtain approval from the IRBs for all subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study. The investigators will notify the reviewing IRBs of protocol violations and SAEs as specified in the relevant sections of the protocol. The results of the study will be shared with the IRBs.

14.2 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.
Consent forms will be approved by all participating IRBs. The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. The informed consent process will be documented in the subject’s research chart, as required by 21 CFR 312.62. The ICF will be signed (or fingerprinted) and personally dated by the subject and the person who conducted the informed consent discussion. The original signed ICF will be retained in the subject’s chart and a signed and dated copy will be provided to the subject. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

14.2.1 Community Permission in Mali

Community permission will be obtained from village elders, family heads, and other community members after explanation and discussion of the study (Diallo et al., 2005). The community permission process goes through the following steps:

- Study investigators/personnel explain the study to village leaders, including the village chief, family heads, women association, and elders.

- The village leaders then discuss the study with family heads and community members and relay any additional questions or concerns they may have to study personnel.

- The study and the informed consent process are explained in detail to heads of families by study investigators/personnel.

At the time of community permission, the need for both husband and wife to agree to avoid pregnancy for the specified period if a wife chooses to volunteer will also be addressed.
The individual informed consent process and form will be translated into French. The study team conducts careful word-for-word review of the study consent form, and will translate the consent orally into local languages, as the majority of potential study subjects do not read or speak French. Verification that the oral translations are accurate and that the potential subjects understand the contents of the informed consent form will be done by an independent witness who is not a member of the study team.

14.2.2 **Individual Informed Consent in Mali**

Local households and families will be invited to come to the study clinic for review of the informed consent, and if the subject agrees to participate, the subject will sign or fingerprint the consent form.

At the consenting visit, the subject will read the consent form, or have it explained in cases of illiteracy. Individuals in each family will be separately consented and not all individuals from a household need to participate.

Subjects will be encouraged to ask questions, and then take a multiple-choice questionnaire (true/false) to evaluate consent comprehension. All incorrect responses will be reviewed with the subject, and he or she must verbalize understanding of all incorrect responses. A score of ≥80% correct is required for enrollment. For subjects scoring less than 80%, study staff may choose to review study details again with subject and reassess comprehension with a repeat Malaria Comprehension Exam. At the discretion of the investigator, any subject whose comprehension is questionable, regardless of score, may be excluded from enrollment.

The Malaria Comprehension Exam will be translated into French and administered orally in the native dialect in the case of potential subjects who cannot read. Study staff will use incorrect answers from the questionnaire to identify those areas of the study procedures that need further review with subject. This will help ensure that the subject has sufficient understanding of the study before enrollment. Subjects unable to read will place a fingerprint in the place of a signature. In addition, an independent witness will sign the consent form to attest that the consent was fully explained and all questions were answered.

14.3 **Subject Confidentiality**

Subjects will not be identified in any publicly released reports of this study. All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator. The investigator will inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number in order to maintain subject confidentiality. All records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, FDA, NIAID, OHRP, or the sponsor's designee.

15 POTENTIAL RISKS AND BENEFITS

For any clinical trial, the risk for potential subjects should be weighed against the benefit. As no direct benefit to trial subjects is likely, benefits are considered in the context of possible public health gains that might occur as a result of this study.
Risks to the subjects are associated with venipuncture, immunization, or drug administration. These risks are outlined below:

15.1 Venipuncture

Risks occasionally associated with venipuncture include pain, bruising, bleeding, and infection at the site of venipuncture, lightheadedness, and rarely, syncope.
The total amount of blood collected are well within the American Association of Blood Banks recommendations, and the current NIH guidelines, and will not compromise these otherwise healthy subjects (Howie, 2011). Any minor bruising, local tenderness or pre-syncopal symptoms, or rarely, infection associated with venepuncture, will be documented as AEs.

15.2 DVI Immunizations (either PfSPZ Vaccine or Normal Saline)

Possible local injection reactions resulting from DVI include pain, swelling, erythema, induration, limitation of limb movement for several days, lymphadenopathy, bruising, or pruritus at the injection site. Systemic reactions such as fever, chills, headache, fatigue, malaise, myalgia, and joint pain may also occur, and may range from mild to severe. These side effects will be monitored but are generally mild and self-limiting.
As with any investigational product, immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible. There is a theoretical possibility of risks about which we have no present knowledge. Subjects will be informed of any such risks should further data become available.
Subjects may be asked to defer routine immunization until 14 – 28 days following vaccination.

15.3 PfSPZ Vaccine

Based on vaccinations administered thus far, the most frequent AEs reported have been headache and malaise, with the majority mild to moderate in nature. Laboratory abnormalities seen include mild, transient, and asymptomatic change in liver enzymes (ALT and/or aspartate aminotransferase [AST]), WBC (leukopenia), and absolute granulocyte counts.

Possible local reactions include pain, swelling, erythema, induration, limitation of limb movement for several days, lymphadenopathy, or pruritus at the injection site. Systemic reactions such as fever, chills, headache, fatigue, malaise, myalgia, and joint pain may also possibly occur, with some reactions moderate or severe.

Other than mild and transient local (site of administration) reactions, the listed adverse reactions remain theoretical.

In Equatorial Guinea two serious adverse events possibly related to PfSPZ Vaccine have been described. One volunteer who received PfSPZ Vaccine had a miscarriage at 10 weeks after getting pregnant while participating in a PfSPZ Vaccine trial. On vaccination day, the subject had a negative urine pregnancy test. She was started on contraceptive measures on the same day she received the vaccine and was advised to additionally use a barrier method.

Miscarriages frequently occur without known causes and although it is unlikely the vaccine caused the miscarriage, the temporal relationship meant this was a possibility. All women of child-bearing age are required to take birth control measures as specified within the

protocol and/or consent form to avoid getting pregnant while participating in trials of PfSPZ-based products. In this protocol, volunteers must use two forms of contraception and must have started a hormonal method at least 21 days prior to receipt of Sanaria® PfSPZ Vaccine injection.

Another volunteer, a 15-year-old boy who received PfSPZ Vaccine, had a generalized seizure 3 ½ hours after receiving his third dose. The boy fully recovered from the seizure. The EEG showed that he was predisposed to having seizures. It is unlikely the vaccine caused the seizure, but like all vaccines, PfSPZ Vaccine causes an immune response in the body which may increase the chance that those individuals predisposed to seizures experience a seizure.

As with any infusion, immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible. There is a theoretical possibility of risks about which we have no present knowledge. Subjects will be informed of any such risks should further data become available.

Subjects may be asked to defer routine immunization (such as influenza) until 14 – 28 days following vaccination.

15.4 Normal Saline

The amount of normal saline used in this study is small (< 1 mL) and has been well tolerated, including in previous PfSPZ Vaccine studies. Overall, most AEs reported with the use of normal saline have been reactions that may have occurred because the technique of administration and have been local injection reactions as noted above in **Section 15.2**.

15.5 Antimalarial Medication

Subjects will be treated with a registered, oral, proven, and highly efficacious treatment during the course of this study.

All antimalarials considered for this trial have an excellent safety profile and are generally well tolerated in the dosing regimens to be used, although common side effects (e.g., nausea, vomiting, diarrhea, headaches) are anticipated to occur in some subjects. Subjects in the study will be closely monitored for adverse drug reactions and where possible, attempts will be made to minimize anticipated side effects. Specific toxicities for Coartem are noted below and further outlined in the IB and the package insert for each drug

Potential subjects with known sensitivity or contraindications to the antimalarial administered in this study are excluded from participation. The most common / important side effects are listed below for each drug. A more comprehensive list of possible side effects is provided in the associated Package Insert.

15.5.1 Coartem® (Artemether/Lumefantrine)

For artemether/lumefantrine the most commonly reported side effects in adults are: headache, anorexia, dizziness, asthenia, arthralgia and myalgia. Generally, these effects do not require discontinuation of the drug.

Artemether/lumefantrine has an acceptable safety profile. Individuals who may have any contraindication for the use of this drug (e.g. prolonged QTc or taking other medications that can prolong QTc, history of myocardial infarction) will be excluded at screening. The most common side effects (i.e., >30%) in adults are: headache, anorexia, dizziness, asthenia, arthralgia, and myalgia. Discontinuation of artemether/lumefantrine due to AE is rare (0.2%) in adults. Rare but

serious hypersensitivity reactions (urticarial and angioedema) and skin reactions (bullous eruption) have been reported post marketing.

Artemether/lumefantrine is a Category C pregnancy drug. Thus, all female participants will undergo pregnancy testing prior to receipt of the investigational dose of artemether/lumefantrine at the start of the study. Also, per the package insert, artemether/lumefantrine may decrease the efficacy of hormonal birth control, so female volunteers who are on hormonal birth control will be counseled about back up pregnancy prevention methods.

For complete artemether/lumefantrine safety information, including less commonly reported side effects, please refer to the Package Insert for Coartem that is provided.

15.6 Cardiac Abnormalities

There is no specific known cardiac risk for healthy subjects associated with experimental malaria challenge/infection or the antimalarial drugs at the proposed dosing used in this study. Likewise, cardiac abnormalities in uncomplicated clinical malaria are extremely rare and routine cardiac monitoring of subjects with severe malaria is not required even in subjects receiving treatment with antimalarials with known effects on cardiac electrical conduction (Bethell et al., 1996; Bregani, Tien, Cabibbe, Figini, & Manenti, 2004; Ehrhardt et al., 2005).

A CHMI subject participating in a malaria vaccine trial in the Netherlands had an unexplained cardiac event following receipt of an investigational vaccine, malaria challenge, infection and treatment with artemether/lumefantrine (Nieman et al., 2009). It is thought the temporal association of the event to malaria challenge was likely circumstantial (Lyke et al., 2010). The definitive etiology of the event remains unknown and the subject recovered without sequelae.

A second cardiac event in a malaria vaccine trial participant in the Netherlands was reported (van Meer et al., 2014). The trial subject received a different test malaria vaccine than the subject reported in 2009. The subject was observed on day 13, after CHMI, to have changes in a blood test suggesting a heart muscle problem. Later that day the subject experienced chest pain and reported a heavy feeling in his left arm. After further evaluation the subject was diagnosed with myocarditis. It is not known whether this illness was related to malaria infection, the test vaccine that the subject received, the medicine used to treat malaria, a viral infection unrelated to the study or something else. Myocarditis resolved without treatment.

Cardiac events, such as coronary vasospasm or myocarditis, are not associated with uncomplicated natural *P. falciparum* infection or experimental infection in the cumulative experience in over 1300 volunteers at three centers worldwide (Ehrhardt et al., 2005). However, in order to minimize the potential risk for cardiac abnormalities during the study, cardiac risk assessment will be conducted as part of the screening process based on screening ECG (subjects with clinically significant abnormalities on ECG will be excluded from the study) and targeted cardiac medical and family history questioning at screening.

15.7 Pregnancy

Malaria infection can have adverse effects on both the pregnant mother and fetus. Women who are pregnant, nursing or plan to become pregnant during the study are excluded from the study. Pregnancy testing is performed during screening and throughout the study, including prior to each PfSPZ Vaccine or Placebo vaccine via DVI and prior to artemether/lumefantrine dosing. If clinical history indicates recent sexual activity that may lead to pregnancy, a serum β-HCG test will be performed and results reviewed by a study investigator prior to the subject continuing in

the study. Pregnancy prevention counseling and compliance is reinforced at every clinic visit (see **Appendix A**) throughout the main study.

If a subject becomes pregnant during the Study, the subject will not be withdrawn from the study but no further investigational products will be administered. The Sponsor and NIAID IRB/FMPOS EC will be notified of the pregnancy. We will confirm the pregnancy by urine (or serum) pregnancy test. The subject will be advised to follow up for antenatal care per Mali Ministry of Health guidelines, this may include receipt of intermittent preventive treatment for prevention of malaria in pregnancy.

The subject will then be provided with an alternative study schedule which will include only obtaining samples for malaria infection evaluation and smaller amount of blood sample for immunogenicity assessment at their scheduled visits and the option to provide a 4mL sample for parasite purification if blood smear positive as outlined in **Appendix A**.. This study schedule may be altered at the investigator's discretion if any concern that the blood draws would impact the expecting the health of the mother or the fetus.

Pregnant subjects who develop clinical malaria will be treated following Mali National Policy on Malaria Control guidelines. Uncomplicated malaria in the 1st trimester is treated with quinine and during 2nd and 3rd trimesters, treatment is with Coartem® [artemether/lumefantrine.] Complicated malaria in pregnancy is treated similarly to non-pregnant patients.

15.8 Risk to the Community

PfSPZ Vaccine is not known to cause any risk to the community.

15.9 Benefits

Subjects will not receive any direct benefit from participation in this study unless the immunization provides long term anti-malarial immunity, which is unknown at this time. It is hoped that information gained in this study will contribute to the development of a safe and effective malaria vaccine.

16 COMPENSATION

Subjects will be given in kind (such as rice and/or millet) or cash equivalent, that can be given in multiple installments as outlined in **Table 9**, to compensate for the time taken to come to the study clinic for study-related visits. Preferred compensation will be decided in consultation with village elders, but case-by-case exceptions to receive the cash equivalent may be acceptable. The Mali EC recommends compensating the study subject for their time lost for study procedures. The amount is equivalent to USD $6 for each scheduled visit with blood drawn and equivalent to USD $3 for each scheduled visit without blood drawn. For unscheduled visits that request research labs to be drawn at that time, such as positive blood smear visits during the malaria transmission season, subjects will be compensated USD $6.

Table 9: Estimated Compensation for the Study

Disbursements to subjects of compensation will be made periodically throughout the trial.

Study Group(s)	Study Activity	US Dollar Equivalent in Rice or Millet Dispensed (Local Currency [CFA]

1, 2, 3a, 3b	Screening & Enrollment	$12 = 6,500 F CFA
1, 3a	Completion of Vaccination #1 & #2	$81 = 43,600 F CFA
2, 3b	Completion of Vaccination #1 & #2	$33 = 17,700 F CFA
1, 2, 3a, 3b	Completion of Vaccination #3 & follow up	$90 = 48,400 F CFA
1, 2, 3a, 3b	AL dosing before vaccination #3	$6 = 3,200 F CFA
1, 2, 3a, 3b	Screening & Pre-Injection #4 visits	$24 = 13,000 F CFA
1, 2, 3a, 3b	Completion of Injection #4 & follow up	$90 = 48,400 F CFA

*Assuming currency exchange rate of USD $1= 538 CFA

17 DATA HANDLING AND RECORD KEEPING

17.1 Data Capture and Management

In Mali, the study data will be collected on paper CRFs and then put into a study specific DataFax electronic database or entered directly into DataFax electronic database. Data from CRFs will be collected directly from subjects during study visits and telephone calls. CRFs (paper or electronic) will be used as source. Any type of corrections to paper CRFs must be initialed and dated by the person making the correction. Any type of corrections to electronic CRFs will be tracked electronically with time, date, individual making the correction, and what was changed. All CRFs should be reviewed by the Investigator or designee and signed as required with written or electronic signature.

Data entry will be performed by authorized individuals. Corrections to the electronic data systems will be tracked electronically (password protected or through an audit trail) with time, date, individual making the correction, and what was changed. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

17.2 Types of Data

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Complete source documentation (laboratory test reports, hospital or medical records, progress notes, observations, etc.) is required for every study subject for the duration of the study. Source documentation will be made available for review or audit by the Sponsors, or their designees and any applicable Federal authorities.

17.3 Retention of Study Records

The investigator is responsible for retaining all essential documents listed in the ICH GCP Guideline. Study records will be maintained by the PI for a minimum of three years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID and Sanaria, Inc with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO or Sanaria, Inc.

17.4 Protocol Revisions

No revisions to this protocol will be permitted without documented approval from the IRBs that granted the original approval for the study. Any change to the protocol will be submitted to the sponsor for review and then to the participating IRBs as a protocol amendment; changes not affecting risk to subjects may request an expedited review. In the event of a medical emergency, the investigator shall perform any medical procedures that are deemed medically appropriate and will notify the IND sponsor of all such occurrences.

18 APPENDICES

Appendix A: Study Schedule

Appendix A: Clinic and Laboratory Procedures For Arm 1 and 3a																																						
		Clinic visits	1	2	3	4	5	6	7	8	9	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Unscheduled -- Blood Smear
		Study Day	-56	-14	1	2	4	8	15	29	43	57	58	60	64	71	85	99	113	114	116	120	127	141	155	169	183	197	211	225	239	253	267	295	N/A
		Days post-PfSPZ Vaccine	-56	-14	0	1	3	7	14	28	42	0	1	3	7	14	28	42	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	182	N/A
		Weeks post-PfSPZ Vac #1	-8	-2	0			1	2	4	6	8			9	10	12	14	16			17	18	20	22	24	26	28	30	32	34	36	38	42	N/A
		Visit windows (days)		"-7" "+14"	±2	0	±1	±2	±3	±5	-5	±14	0	±1	±2	±3	±5	-7	±14	0	±1	±2	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	+42	
			Screen	Enroll	PfSPZ Vac #1	Safety F/U	Safety F/U	Safety F/U	Safety F/U	Safety F/U	Safety F/U; Pre-Vac	PfSPZ Vac #2	Safety F/U	Safety F/U	Safety F/U	Safety F/U	Safety F/U	Pre-Vac; AL	PfSPZ Vac #3	Safety F/U	Safety F/U	Safety F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Unblinding visit	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																																						
Complete medical history/ physical			X																																		
Informed consent			X																															X[2]		
Malaria Comprehension Exam			X																																		
Pre-test/Post-test HIV counseling			X																																		
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	
AL dose																		X																				
PfSPZ Vaccine (Arm 1 only)[1]					X							X							X																		
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																																				
Screening/Safety Labs and procedures																																						
CBC with differential	MRTC CAP lab/UB/LPD	EDTA	2	2			2	2						2	2			2	2		2		2		2			2				2						
ALT, Cr		SST	3	3			3	3						3	3			3	3		3		3		3			3				3						
Hepatitis B, C, HIV testing; sickle cell screening		SST	5																																			
Urine dipstick or UA		Urine Container	X																																			
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X	X							X						X	X																			
ECG	NIH/Mali Cardio	N/A	X																																			
Malaria Infection Assays																																						
qPCR	MRTC CAP lab/LMIV	EDTA		0.5	0.5				0.5			0.5				0.5		0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5			0.5	
Peripheral Blood Smear		EDTA		0.5	0.5		0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5	0.5	0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5				
Research Assays																																						
Humoral Assays	MRTC/ LMIV	SST		10												2.5		5					5	2.5				2.5				2.5						
Cellular Assays		NaHep		10												10		10				10	10	10				10				10						
Ex Vivo Assays		EDTA		0.5			0.5									0.5		0.5				0.5	0.5	0.5				0.5				0.5					0.5	
Parasite Purification (CF11)		EDTA																																			4	
Transcriptional Assays		PaxGENE		1														1			1	1		1								1						
Daily total			10	27.5	1	0	6	5.5	1	0.5	0.5	1	0	5.5	5.5	14	0.5	22.5	6	0	6	12.5	21.5	15	6	1	1	19	1	1	1	20	1	0		5	
Study cumulative total			10	37.5	38.5	38.5	44.5	50	51	51.5	52	53	53	58.5	64	78	78.5	101	107	107	113	125.5	147	162	168	169	170	189	190	191	192	212	213	213			
[1] Arm 3a receives Normal Saline DVI																																						
[2] Booster phase informed consent																																						

Appendix A: Clinic and Laboratory Procedures For Arm 2 and 3b

		Clinic visits	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	Unscheduled – Blood Smear Positive
		Study Day	-56	-14	1	2	4	8	9	11	15	22	29	30	32	36	43	57	71	85	99	113	127	141	155	169	183	211	N/A
		Days post-PfSPZ Vaccine	-56	-14	0	1	3	0	1	3	7	14	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	182	N/A
		Weeks post-PfSPZ Vac #1	-8	-2				1			2		4				6	8	10		14		18		22		26	30	N/A
		Visit windows (days)		"-7" "+14"	±2	0	±1	±1	0	±1	±2	±3	±2	0	±1	±2	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	+42	
			Screen	Enroll	PfSPZ Vac #1	Safety F/U	Safety F/U; Pre-Vac	PfSPZ Vac #2	Safety F/U	Safety F/U	AL	Pre-vac	PfSPZ Vac #3	Safety F/U	Safety F/U	Safety F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Final & Unblinding/ Clinic visit	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																													
Complete medical history/ physical			X																									
Informed consent			X																								X[2]	
Malaria Comprehension Exam			X																									
Pre-test/Post-test HIV counseling			X																									
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AL dose											X	X																	
PfSPZ Vaccine (Arm 2 only)[1]					X			X					X																
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																											
Screening/Safety Labs and procedures																													
CBC with differential	MRTC CAP lab/UB/LPD	EDTA	2	2			2				2		2		2		2		2			2				2			
ALT, Cr		SST	3	3			3				3		3		3		3		3			3				3			
Hepatitis B, C, HIV testing; sickle cell screening		SST	5																										
Urine dipstick or UA		Urine Container	X																										
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X	X			X			X		X																
ECG	NIH/Mali Cardio	N/A	X																										
Malaria Infection Assays																													
qPCR	MRTC CAP lab/LMIV	EDTA		0.5	0.5			0.5		0.5	0.5		0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Peripheral Blood Smear		EDTA		0.5	0.5		0.5	0.5		0.5	0.5		0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
Research Assays																													
Humoral Assays	MRTC/ LMIV	SST		10							5						5	2.5				2.5				2.5			
Cellular Assays		NaHep		10							10					10	10	10				10				10			
Ex Vivo Assays		EDTA		0.5			0.5				0.5					0.5	0.5	0.5				0.5				0.5			0.5
Parasite Purification (CF11)		EDTA																											4
Transcriptional Assays		PaxGENE		1							1				1			1								1			
Daily total			10	27.5	1	0	6	1	0	1	22.5	0	6	0	6	11.5	21.5	15	0	1	1	19	1	1	1	20	1	1	5
Study cumulative total			10	37.5	38.5	38.5	44.5	45.5	45.5	46.5	69	69	75	75	81	92.5	114	129	129	130	131	150	151	152	153	173	174	175	
[1] Arm 3b receives Normal Saline DVI																													
[2] Booster phase informed consent																													

Appendix A: Clinic and Laboratory Procedures For Arms 1 & 3a - Booster (Vaccination # 4)																							
		Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Unscheduled -- Blood Smear Positive
		Study Day	323	351	362	372	379	380	382	386	393	407	421	435	449	463	477	491	505	519	533	561	N/A
		Days post-PfSPZ Vac #4	-56	-28	-17	-7	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	182	N/A
		Weeks post-PfSPZ Vac #1	46	50			54					58		62		66		70		74		80	N/A
		Visit windows (days)	-14/+21	-14/+21	-7/+2	±3	-7/+14	+1	+1	±2	±3	±3	±5	±5	±5	±5	±5	±5	±5	±5	±5	±14	
			Clinic f/u	Booster Screen	AL	Safety F/U	PfSPZ Vac #4[1]	Safety F/U	Safety F/U	Safety F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Unblinding visit	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																							
Complete medical history/ physical				X																			
Malaria Comprehension Exam				X																			
Pre-test/Post-test HIV counseling[2]				(X)																			
Pregnancy Prevention Counseling				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Interim clinical evaluation			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AL dose					X																		
PfSPZ Vaccine (Arm 1 only)[1]							X																
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																					
Screening/Safety Labs and procedures																							
CBC with differential	MRTC CAP lab/LJB/LPD	EDTA		2		2			2		2		2			2				2			
ALT, Cr		SST		3		3			3		3		3			3				3			
Hepatitis B, C, HIV testing[2]		SST		(5)																			
Urine dipstick or UA		Urine Container																					
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep		X	X		X																
ECG[2]	NIH/Mali Cardio	N/A		(X)																			
Malaria Infection Assays																							
qPCR	MRTC CAP lab/LMIV	EDTA	0.5	0.5	0.5	0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Peripheral Blood Smear		EDTA	0.5	0.5	0.5	0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Research Assays																							
Humoral Assays	MRTC/ LMIV	SST		10	5						5	2.5				2.5				2.5			
Cellular Assays[3]		NaHep		10	10					10	10	10				10				10			
Ex Vivo Assays		EDTA			0.5						0.5					0.5				0.5			
Parasite Purification (CF11)		EDTA																					(4)
Transcriptional Assays		PaxGENE		1	1				1			1								1			
Daily total			1	27	17.5	6	1	0	6	11	21.5	14.5	6	1	1	19	1	1	1	20	1	0	
Study cumulative total			1	28	45.5	51.5	52.5	52.5	58.5	69.5	91	105.5	111.5	112.5	113.5	132.5	133.5	134.5	135.5	155.5	156.5	157	

[1] Arm 3a receives Normal Saline DVI

[2] Procedure to be completed ONLY if clinically indicated

[3] If a woman becomes pregnant after 4th vaccination and remains in the study then only 5ml will be drawn if no other contraindications for blood draw

Appendix A: Clinic and Laboratory Procedures For Arms 2 & 3b - Booster (Vaccination # 4)

		Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Unscheduled -- Blood Smear Positive
		Study Day	239	267	278	288	295	296	298	302	309	323	337	351	365	379	393	407	421	435	449	477	N/A
		Days post-PfSPZ Vac #4	-56	-28	-17	-7	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	182	N/A
		Weeks post-PfSPZ Vac #1	34	38			42					46		50		54		58		62		68	N/A
		Visit windows (days)	-14/+21	-14/+21	-7/+2	±3	-7/+14	+1	+1	±2	±3	±3	±5	±5	±5	±5	±5	±5	±5	±5	±5	±14	
			Clinic f/u	Booster Screen	AL	Safety F/U	PfSPZ Vac #4[1]	Safety F/U	Safety F/U	Safety F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Malaria F/U	Unblinding visit	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																							
Complete medical history/ physical				X																			
Malaria Comprehension Exam				X																			
Pre-test/Post-test HIV counseling[2]				(X)																			
Pregnancy Prevention Counseling				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Interim clinical evaluation			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AL dose					X																		
PfSPZ Vaccine (Arm 2 only)[1]							X																
LABORATORY PROCEDURES	**Designated Laboratory**	**Tube Type**																					
Screening/Safety Labs and procedures																							
CBC with differential	MRTC CAP lab/UB/LPD	EDTA		2		2			2		2		2			2				2			
ALT, Cr		SST		3		3			3		3		3			3				3			
Hepatitis B, C, HIV testing[3]		SST		(S)																			
Urine dipstick or UA		Urine Container																					
Pregnancy Test (females only)		Container or Lithium Hep		X	X		X																
ECG[2]	NIH/Mali Cardio	N/A		(X)																			
Malaria Infection Assays																							
qPCR	MRTC CAP lab/LMIV	EDTA	0.5	0.5	0.5	0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Peripheral Blood Smear		EDTA	0.5	0.5	0.5	0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Research Assays																							
Humoral Assays	MRTC/ LMIV	SST		10	5						5	2.5				2.5				2.5			
Cellular Assays[3]		NaHep		10	10					10	10	10				10				10			
Ex Vivo Assays		EDTA			0.5						0.5					0.5				0.5			
Parasite Purification (CF11)		EDTA																					(4)
Transcriptional Assays		PaxGENE		1	1				1			1								1			
Daily total			1	27	17.5	6	1	0	6	11	21.5	14.5	6	1	1	19	1	1	1	20	1	0	
Study cumulative total			1	28	45.5	51.5	52.5	52.5	58.5	69.5	91	105.5	111.5	112.5	113.5	132.5	133.5	134.5	135.5	155.5	156.5	157	

[1] Arm 3b receives Normal Saline DVI

[2] Procedure to be completed ONLY if clinically indicated

[3] If a woman becomes pregnant after 4th vaccination and remains in the study then only 5ml will be drawn if no other contraindications for blood draw

Appendix B: Toxicity Table

Local Reactogenicity Grading

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain/Tenderness/Pruritus	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Erythema/Redness/Bruising[1]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling[2]	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

[1] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[2] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Sign AE Grading

Vital Signs[1]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever[2] (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - bpm	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - bpm[3]	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock

[1] Subject should be at rest for all vital sign measurements.
[2]Oral temperature; no recent hot or cold beverages or smoking.
[3]When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic AE Grading

Systemic adverse events[1]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Feverish	No interference with activity	Some interference with activity not requiring medical intervention or use of 1-2 doses of antipyretics	Prevents daily activity or use of >2 doses of antipyretics in 24 hours	ER visit or hospitalization
Chills/Rigors	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity	ER visit or hospitalization for hypotensive shock
Sweats	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity	ER visit or hospitalization
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue/ Malaise	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity	ER visit or hospitalization
Back Pain	No interference with activity	Some interference with activity; use of 1-2 doses of medication	Prevents daily activity	ER visit or hospitalization
Chest Pain (non-musculoskeletal)	Transient (< 24 hours) or intermittent chest pain with no or minimal interference	Persistent chest pain resulting in greater than minimal interference with usual activities	Persistent chest pain resulting in inability to perform usual activities secondary to chest pain	ER visit or hospitalization
Myalgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization

Systemic adverse events[1]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Arthralgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Nausea/ Vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Abdominal Pain	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Urticaria/Rash	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization
Edema	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity	ER visit or hospitalization
Pruritus	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization

[1] For other symptoms not listed, they should be graded as outlined in **Section 11.3.1**

***Adapted from Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine clinical Trials.

Mali Laboratory AE Grading
Hematology/Chemistry

Hematology and Biochemistry values[1, 2]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male) - gm/dL	9.5 – 10.3	8.0 – 9.4	6.5 – 7.9	< 6.5 and / or requiring transfusion
Hemoglobin (Female) gm/dL	8.0 – 9.0	7.0 – 7.9	6.0 – 6.9	< 6 and /or requiring transfusion
WBC Increase – 10^3/µL	11.5 – 15.0	15.1 – 20.0	20.1 – 25.0	> 25.0
WBC Decrease - 10^3/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0 with fever
Granulocyte or Neutrophil Decrease - 10^3/µL	0.8 – 1.0	0.5 – 0.7	< 0.5	< 0.5 with fever
Platelets Decreased – 10^3/µL	100 – 110	70 – 99	25 – 69	< 25
Creatinine (Male) µmol/L	124.00 – 150.99	151.00 – 176.99	177.00 – 221.00	> 221.00 and requires dialysis
Creatinine (Female) µmol/L	107.00 – 132.99	133.00 – 159.99	160.00 – 215.99	> 216.00 and requires dialysis
Liver Function Tests –ALT U/L	75.0 – 150.9	151.0 – 300.9	301.0 – 600.0	> 600.0

[1]The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
[2]The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Mali Adult Normals
Chemistry

Serum[1]	Reference Range
Creatinine (Female) - µmol/L	< 72
Creatinine (Male) - µmol/L	48 – 98
ALT – U/L	< 41

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Hematology

Hematology[1]	Reference Range
Hemoglobin (Female) - gm/dL	9.1 – 13.8
Hemoglobin (Male) - gm/dL	10.8 – 15.8
WBC – $10^3/\mu L$	3.6 – 9.0
Absolute Granulocyte or Neutrophil Count - $10^3/\mu L$	1.3 – 4.4
Absolute Lymphocyte Count - $10^3/\mu L$	1.3 – 4.4
Platelet Count (Female)- $10^3/\mu L$	144 – 413
Platelet Count (Male)- $10^3/\mu L$	114 – 335

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Urine Dip/Urinalysis

Urine[1]	Reference Ranges
Protein	None
Blood (microscopic) – red blood cells per high power field (RBC/HPF)	None or Trace < 1

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Appendix C: References

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