Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Immunogenicity, and Protective Efficacy of Radiation Attenuated *Plasmodium falciparum* NF54 Sporozoites (PfSPZ Vaccine) During Malaria Transmission Season in Healthy African Adult Women of Childbearing Potential in Mali

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D. Makes decisions about subject eligibility

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
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
CMF	Clinical Manufacturing Facility
CoA	Certificate of Analysis
Cr	creatinine
CRF	case report forms
CSP	circumsporozoite protein
DNA	Deoxyribonucleic acid
DOT	directly observed therapy
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
HELLP	Hemolysis, elevated liver enzymes, low platelets
HIV	human immunodeficiency virus
HRPP	Human Research Protection Program
HSA	human serum albumin
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonization
ICS	Intracellular cytokine staining

ID	Intradermal administration
IFA	immunofluorescence assay
IND	Investigational New Drug Application
IPTp	intermittent preventive treatment during pregnancy
IRB	Institutional Review Board
IRS	Indoor residual spraying
ISI	Inhibition of sporozoite invasion assay
ISM	Independent Safety Monitor
ITN	insecticide-treated bed net
ITT	intention to treat
IV	intravenous
LBW	Low birth weight
LMIV	Laboratory of Malaria Immunology and Vaccinology
LNVP	liquid nitrogen vapor phase
MLE	Maximum likelihood estimation
MRTC	Malaria Research and Training Center
NASBA	Nucleic acid sequence-based amplification
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
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
PfCSP	*Plasmodium falciparum* circumsporozoite protein
PfSPZ	*Plasmodium falciparum* sporozoites
PfSPZ Challenge	viable *Plasmodium falciparum* sporozoites produced by Sanaria, Inc.
PfSPZ Vaccine	radiation attenuated *Plasmodium falciparum* sporozoites produced by Sanaria, Inc.
PI	Principal Investigator
PM	placental malaria
qPCR	quantitative polymerase chain reaction
QTc	QT (corrected) interval
RDT	rapid diagnostic test
RNA	Ribonucleic acid
SAE	serious adverse event
SAR	suspected adverse reaction
SC	Subcutaneous

SMC	Seasonal malaria chemoprophylaxis
SNP	single-nucleotide polymorphism
SOP	standard operating procedure
SPZ	Sporozoites
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
WOCBP	Women of child bearing potential
WRAIR	Walter Reed Army Institute of Research

PROTOCOL SUMMARY

Full Title:	Randomized, Placebo-Controlled, Double-Blind Study to Assess Safety, Immunogenicity, and Protective Efficacy of Radiation Attenuated *Plasmodium falciparum* NF54 Sporozoites (PfSPZ Vaccine) During Malaria Transmission Season in Healthy African Adult Women of Childbearing Potential in Mali
Short Title:	PfSPZ Vaccine in WOCBP
Clinical Phase:	Phase 2
IND Sponsor:	Sanaria, Inc.
Clinical Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO), NIAID
Conducted by:	Malaria Research and Training Center (MRTC), in collaboration with the Laboratory of Malaria Immunology and Vaccinology (LMIV)
Principal Investigators:	Halimatou Diawara, MD, MPH (MRTC) Patrick Duffy, MD (National Institute of Allergy and Infectious Diseases [NIAID] / National Institutes of Health [NIH]/LMIV)
Vaccine Sample Size:	N=300
Offspring Sample Size:	N~300
Accrual Ceiling:	N=900 (WOCBP + offspring)
Study Population:	Healthy women of child bearing potential (18 to 38 years of age), with planned follow-up post vaccination in future pregnant women + offspring, residents in Ouelessebougou and surrounding villages, Mali
Accrual Period:	Approximately May 2019 to August 2020 Re- enrollment: June 2022 to August 2022
Study Design:	A randomized double blind, placebo-controlled study to assess the safety, tolerability, immunogenicity, and protective efficacy of 1, 8, 29-day PfSPZ Vaccine regimen given at a dose of 9 x10^5 PfSPZ Vaccine or 1.8x10^6 PfSPZ Vaccine or placebo in healthy women of child bearing potential, who are on pregnancy prevention during vaccination, but report plans to become pregnant in the near future. Participants will be randomized into three arms. **Arm 1:** (n= 100) will receive 3 doses of PfSPZ Vaccine (9 x10^5) via direct venous inoculation (DVI) at 1, 8, 29 days.

Arm 2: (n= 100) will receive 3 doses of PfSPZ Vaccine (1.8 x10^6) via DVI at 1, 8, 29 days.

Arm 3: (n=100): will receive 3 doses of normal saline (placebo) injection via DVI at 1, 8, 29 days.

All volunteers will receive antimalarial treatment with artemether/lumefantrine (AL) ~2 weeks prior to 1st (study day -14) and 3rd injection (study day 15).

Participants will be monitored for safety, tolerability, immunogenicity, and malaria infection during the follow-up period.

Participants will also be monitored closely for pregnancy as well post 3rd injection and followed during the course of their pregnancy and for 1 year post delivery during Year 1 (as well as their offspring if pregnancy occurs during Year 1) for safety and malaria infection.

Women who become pregnant during Year 2 of follow-up will be followed during the course of their pregnancy and for ~2 months post-delivery or until the end of the study, whichever is longer. For pregnancies detected in Year 2, subsequent offspring will not be followed beyond delivery.

Women were followed for an additional year in 2020-2021 (no additional product administration) and given continued protective efficacy seen during Malaria Season Year 2, women (pregnant or non-pregnant) who have received at least two vaccinations are being asked to come back to be followed for a 24-week period during the 2022-2023 Malaria Season. No product (AL nor PfSPZ Vaccine nor Normal Saline) will be administered.

Study Duration: Start Date: Approximately May 2019

Primary End Date: Approximately March 2021 (if not pregnant during the course of the study)

Primary End Date: Approximately May 2022 (if WOCBP becomes pregnant during the course of the study)

Extension End Date: Approximately December 2022 to February 2023

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 two doses (9 x10^5 and 1.8 x10^6) at 1, 8, 29 days

Normal saline administered via DVI at 1, 8, 29 days.

Primary Objective:

Safety:

- Assess safety and tolerability of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses (9 x$10^5$and 1.8 x10^6).

Secondary Objectives:

Protective Efficacy:

- Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses (9 x$10^5$and 1.8 x10^6).

Exploratory Objectives:

Protective Efficacy:

- Explore the impact of PfSPZ Vaccine on additional malaria infection endpoints.
- Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses (9 x$10^5$and 1.8 x10^6) for a second malaria transmission season
- Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses (9 x$10^5$and 1.8 x10^6) for another malaria transmission season (durability)

Immunogenicity:

- Characterize and compare host immune responses to malarial antigens, and host proteomic profiles and transcriptomes in African WOCBP 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.

Pregnant Women and Neonates

Safety:

- Assess maternal and neonatal outcomes post receipt of PfSPZ Vaccine primary series

Protective Efficacy:

- Explore the PfSPZ Vaccine protective efficacy and durability of protection in pregnant women.
- Explore the PfSPZ Vaccine protective efficacy and durability of protection in neonates/infants born to PfSPZ vaccinated mothers.

Primary Endpoints:

Safety:

- Incidence of local and systemic adverse events (AEs) graded by severity occurring within 7 days after each vaccine administration.

Secondary Endpoints:

Protective Efficacy:

- *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 over 24 weeks.

Exploratory Endpoints:

Protective Efficacy:

- Measurement and comparison of various malaria infection endpoints, such as PCR, gametocytemia, non-falciparum parasitemia, symptomatic malaria
- *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 approximately 1 year post receipt of 3rd injection for 24 weeks.
- *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 approximately 3 years' post receipt of last injection for 24 weeks.

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.

Pregnant Women and Neonates

Safety:

- Incidence of maternal obstetric outcomes (miscarriage/stillbirth, intrauterine growth restriction, hypertensive diseases in pregnancy, preterm delivery) and neonatal outcomes (neonatal death, low birth weight, small for gestational age, major malformations, and microcephaly)

Protective Efficacy:

- *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) during the pregnancy, during infancy, and starting immediately following 3rd injection
- *P. falciparum* placental infection defined as any positive placental blood smear for *P. falciparum*.

PRECIS

Pregnant women are highly susceptible to *Plasmodium falciparum* malaria, leading to substantial maternal, perinatal, and infant mortality. While malaria vaccine development has made significant progress in recent years, no trials of malaria vaccines have ever been conducted in only women of child bearing potential (WOCBP) or in pregnant women.

PfSPZ Vaccine (Sanaria, Inc) is an advanced malaria candidate being developed for use in pregnant women, owing in part to its highly favorable safety profile. The vaccine is comprised of aseptic, metabolically active, non-replicating, purified, cryopreserved *P. falciparum* sporozoites. In multiple double-blind, placebo-controlled trials, there have been no differences in adverse events between vaccinees versus controls. PfSPZ Vaccine induces immune responses to the sporozoite and liver stages of parasite development in the human host and prevents progression to blood stage parasitemia as well as averting disease sequelae; a compelling rationale to test PfSPZ Vaccine for its benefits in this proposed population.

Sanaria has already achieved vaccine efficacy against homologous and heterogenous parasite populations in endemic areas following three doses of PfSPZ Vaccine in several studies with $9.0x10^5$ and $1.8x10^6$ PfSPZ Vaccine and has explored accelerated regimens, such as 1, 8, 29 days. Accelerated PfSPZ Vaccine regimens such as this could induce protection earlier in pregnancy, minimizing the period at risk and improving pregnancy outcomes over the control group.

Given this, the Malaria Research and Training Center, the Laboratory of Malaria Immunology and Vaccinology National Institute of Allergy and Infectious Diseases, and Sanaria, Inc. propose to initiate testing of the day 1, 8, and 29 dosing regimen of PfSPZ Vaccine in WOCBP, and in subsequent studies, in pregnant women, using doses of $9.0x10^5$ and $1.8x10^6$ PfSPZ Vaccine. This will be the first step in a clinical development plan for PfSPZ Vaccine in WOCBP and pregnant women: 1) safety and efficacy studies in non-pregnant WOCBP (*this trial*), 2) studies of the safety and efficacy of a primary immunization series in all trimesters, and 3) studies to evaluate the safety and efficacy of boosting during pregnancy.

A pregnancy registry study (#17-I-N018) has been ongoing in Mali since 2017 to gain background data on maternal/fetal outcomes in the target population in anticipation of this study. Women who become pregnant during the course of this study, and their offspring, will be followed for maternal clinical outcomes and malaria infection.

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 regarding any hope of eradicating malaria with currently available means, and highlight the urgent need to develop an efficient vaccine to eradicate 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 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 to release the sporozoites. The sporozoites undergo 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 6.1** of this protocol).

1.4.1 Direct Venous Inoculation 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 trials in US and African adults (as summarized in **Table 1**) have been completed to date with reassuring safety results at different PfSPZ vaccine doses (up to $2.7x10^6$ PfSPZ) and dosing regimens. Promising protective efficacy results have also been seen, in particular with an accelerated 1, 8, 29 day regimen as proposed for our study in WOCBP.

Table 1: Chronological Listing of Trials of PfSPZ Vaccine

Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Volunteers**
1. NMRC/UMB CVD (NCT01001650) May 2009 *(completed)*	Phase 1, open-label, dose-escalation with CHMI in USA (**ID, SC only)**	$7.5x10^3$ SC x 4; $7.5x10^3$ ID x 4; $3x10^4$ SC x 4; $3x10^4$ ID x 4; $1.35x10^5$ SC x 4 or 6; $1.35x10^5$ ID x 4 or 6	Malaria-naïve adults: 80
2. VRC 312 (NCT01441167) Oct 2011 *(completed)*	Phase 1, open-label, dose-escalation with CHMI in USA (**IV only)**	$2x10^3$ IV x 2; $7.5x10^3$ IV x 4 or 6; $3x10^5$ IV x 4 or 6; $1.35x10^5$ IV x 4 or 5	Malaria-naïve adults: 40
3. VRC 314 (NCT02015091) Dec 2013 *(completed)*	Phase 1, open-label, dose-escalation, regimen comparison with CHMI in USA (**IV or IM)**	$2.2x10^6$ IM x 4; $1.35x10^5$ IV x 4 + $4.5x10^5$ IV boost; $2.7x10^5$ IV x 3 or 4; $2.7x10^5$ IV x 2 + $4.5x10^5$ IV x 2; $9x10^5$ IV x 3	Malaria-naïve adults: 93
Administration in all following trials by **DVI only**			
4. Mali 1 (NCT01988636) Jan 2014 *(completed)*	Phase 1, randomized, double-blind placebo-controlled* field efficacy in Mali	$1.35x10^5$ + $2.7x10^5$; $2.7x10^5$ x 5	Malaria-exposed adults: 58
5. BSPZV1 (NCT02132299) May 2014 *(completed)*	Phase 1, randomized, double- blind placebo-controlled* with CHMI (by needle and syringe) in Tanzania	$3x10^4$; then $1.35x10^5$; then $2.7x10^5$ $1.35x10^5$ x 5; $2.7x10^5$ x 5	Malaria-exposed adults: 49

Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Volunteers**
6. WRAIR 2080 (NCT02215707) Jun 2014 *(completed)*	Phase 1, open-label, regimen comparison with CHMI in USA	$2.7x10^5$ x 5; $4.5x10^5$ x 3	Malaria-naïve adults: 45
7. EGSPZV1 (NCT02418962) Mar 2015 *(completed)*	Phase 1, open-label, dose-escalation in Equatorial Guinea	$1.35x10^5$; then $2.7x10^5$ $2.7x10^5$ x 3	Malaria-exposed adults: 23
8. BSPZV2 (NCT02613520) Dec 2015 *(completed)*	Phase 1 dose escalation, double-blind, randomized, placebo-controlled* with CHMI (by needle and syringe) in Tanzania	Adults, older children: $9x10^5$ x 3; then $1.8x10^6$ x 3 Younger children, infants: $4.5 x 10^5$ x 3; then $9x10^6$ x 3	Malaria-exposed adults: 12 children: 36 infants: 15
9. Mali 2 (NCT02627456) Jan 2016 *(completed)*	Phase 1 dose escalation with CHMI followed by Phase 2 randomized, double-blind, placebo-controlled* field efficacy in Mali	Ph 1: $4.5x10^5$ x 1; then $9x10^5$ x 1; then $1.8x10^6$ x 3 Ph 2: $1.8x10^6$ x 3	Malaria-exposed adults: 100
10. Burkina Faso 1 (NCT02663700) Apr 2016 *(completed)*	Phase 1 dose escalation followed by Phase 2, randomized, double-blind placebo-controlled* field efficacy in Burkina Faso	Ph 1: $4.5x10^5$ x 2; then $9x10^5$ x 2; then $1.8x10^6$ x 2; then $2.7x10^6$ x 2 Ph 2: $2.7x10^6$ x 3	Malaria-exposed adults: 32
11. Warfighter 2 (NCT02601716) Apr 2016 *(completed)*	Phase 2, open-label, regimen comparison with CHMI in USA	$4.5x10^5$ x 5 (Days 1, 3, 5, 7 and week 16); or $9x10^5$ x 3 (Weeks 1, 9, 17); or $1.8x10^6$ x 3 (Weeks 1, 9, 17); or $2.7x10^6$ x 1 + $9x10^5$ x 2 (Weeks 1, 9, 17)	Malaria-naive adults: 60
12. KSZPV1 (NCT02687373) Jul 2016 *(completed)*	Phase 1 dose escalation followed by Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Kenya	Ph 1 - Older children: $4.5x10^5$ x 1; then $9x10^5$ x 2; then $1.8x10^6$ x 2 Ph 1 - Younger children, infants: $1.35x10^5$ x 1; then $2.7x10^5$ x 1; then $4.5x10^5$ x 1; then $9x10^5$ x 2; then $1.8x10^6$ x 2, all Ph 2 - Infants: $4.5x10^5$, $9x10^5$, or $1.8x10^6$, all x 3	Malaria-exposed children: 64 infants: 352
13. MAVACHE (NCT02704533) Sep 2016 *(completed)*	Phase 1 dose escalation, regimen-condensation and dose number reduction with CHMI in Germany	$9x10^5$ x 3 (Days 1, 8, 29); then $1.8x10^6$ x 2 (Days 1, 8); then $2.7x10^6$ x 2 (Days 1, 8)	Malaria-naive adults: 42
14. EGSPZV2 (NCT02859350) Nov 2016 *(completed)*	Phase 1 dose escalation, randomized double-blind, placebo-controlled* with head-to-head PfSPZ Vaccine and PfSPZ-CVac comparison in Equatorial Guinea	Adults (PfSPZ Vaccine): $2.7x10^6$ x 3 Adults (PfSPZ-CVac): $1x10^5$ x 3 Children, infants (PfSPZ Vaccine): $1.8x10^6$ x 3	Malaria-exposed adults: 52** children: 36 infants: 15

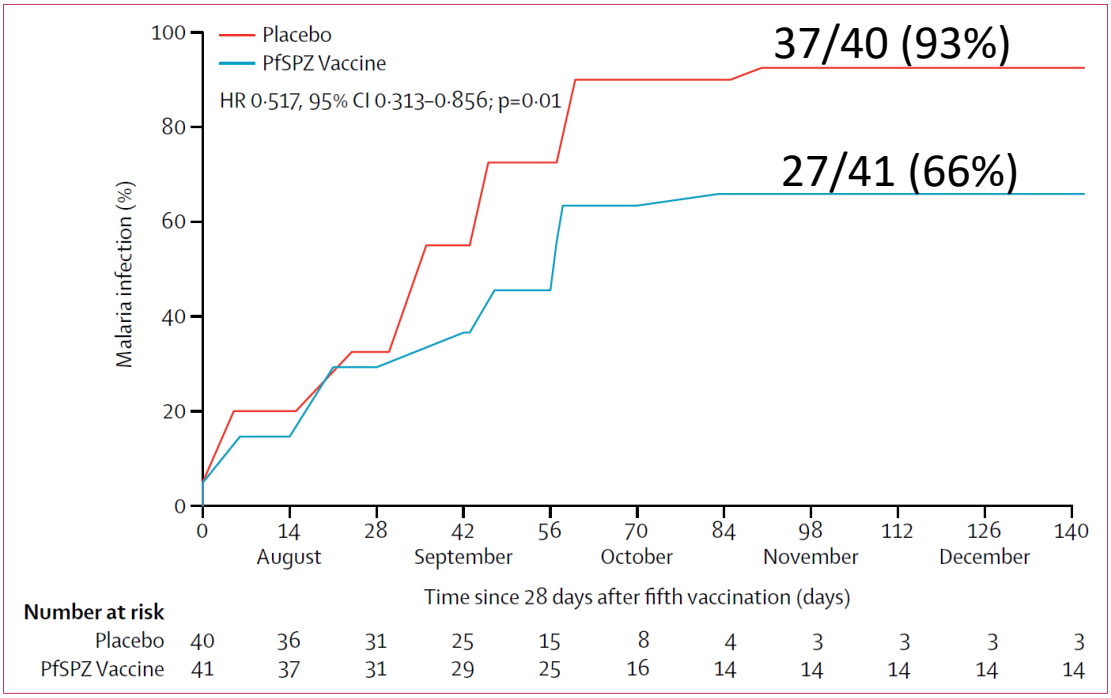
Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Volunteers**
15. BSPZV3a (NCT03420053) *(completed)*	Phase 1 dose escalation, randomized double-blind, placebo-controlled* with CHMI in Tanzania	$4.5x10^5$ x 5 (Days 1, 3, 5, 7 and 29); or $9x10^5$ x 5 (Days 1, 3, 5, 7 and 29)	Malaria-exposed HIV- and HIV+ adults: 21
16. MSPZV3 (NCT03510481) *(ongoing)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Mali	$9x10^5$ x 3 (Days 1, 8 and 29); or $9x10^5$ x 3 (Weeks 1, 9, 17)	Malaria-exposed adults: 210
17. LaSPZV1 (NCT03521973) *(ongoing)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Gabon	$9x10^5$ x 3 (Days 1, 8 and 29)	Malaria-exposed children: 200
18. EGSPZV3 (NCT03590340) *(ongoing)*	Phase 1 double-blind, randomized, placebo-controlled* with CHMI in Equatorial Guinea	$9x10^5$ x 3 (Days 1, 8 and 29); or $9x10^5$ x 5 (Days 1, 3, 5, 7 and 29); or $9x10^5$ x 5 (Days 1, 3, 5, 7 and Week 17); or $9x10^5$ x 4 (Days 1, 3, 5, 7)	Malaria-exposed adults: 104
*The placebo control used in all trials is normal saline; ** 20/52 adult volunteers in EGSPZV2 received PfSPZ-CVac.			
In these trials, dosing schedules have evaluated various intervals between vaccinations ranging from 4 to 16 weeks.			

1.4.3 **Mali (NIAID Protocol 14-I-N010)**

The first, randomized, double-blind, placebo-controlled trial testing vaccine efficacy (VE) against naturally transmitted malaria was conducted in Mali (#4 in **Table 1**) 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). Vaccinations were well-tlerated overall with no serious adverse events (SAEs) reported and most study subjects reporting no local or systemic reactogenicity following vaccination. 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. No significant differences in local or systemic reactogenicity between PfSPZ Vaccine and placebo recipients were noted (Sissoko et al., 2017).

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).

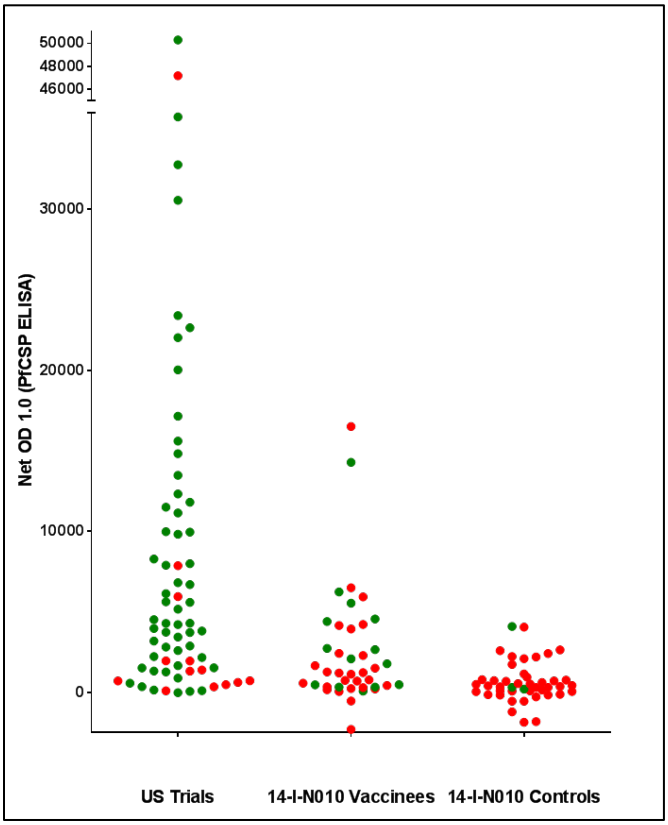
Figure 1: Protective Efficacy: First Positive Blood Smear (BS) Starting 28 Days Post Vaccination #5



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.

1.4.4 Mali (NIAID Protocol 16-I-N004)

Following review of the safety data from a dose escalating pilot phase, 120 subjects healthy Malian adults aged 18-45 years of age 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 (#9 in **Table 1**). 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. 57/60 (95%) PfSPZ Vaccine subjects received all three doses and 55/60 (91.7%) normal saline placebo subjects received all three doses.

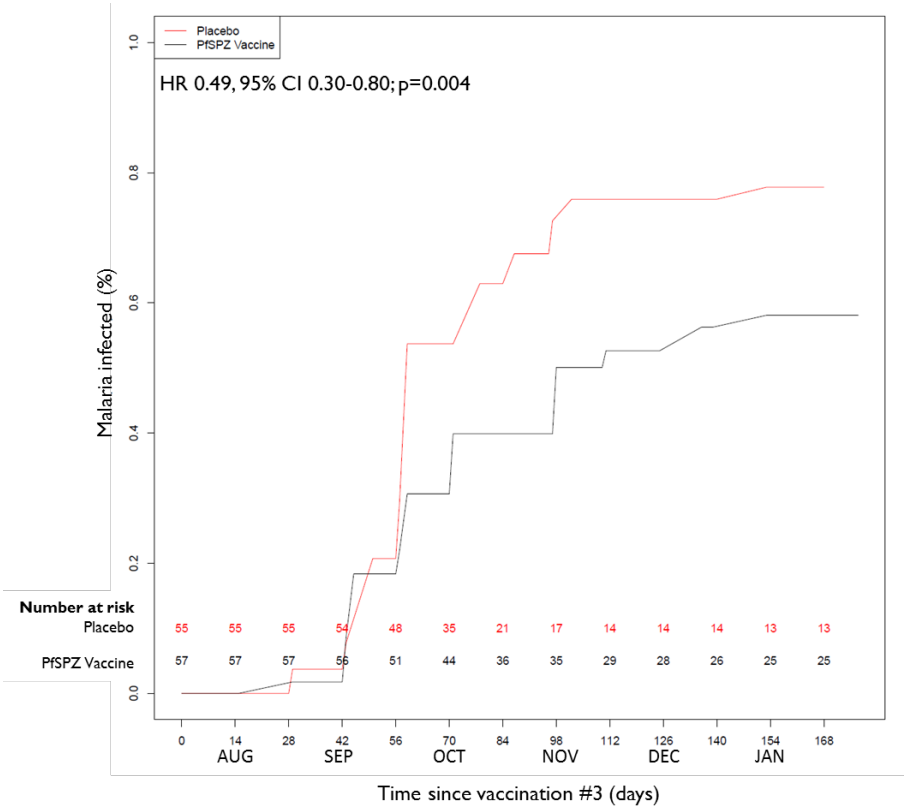
PfSPZ vaccinations were safe and well tolerated except there were three additional unanticipated significantly elevated transaminases (Grade 3 to 4) in three Main Phase participants (1 PfSPZ Vaccine, 2 placebo) at varying stages post vaccination and ASAQ (14 to 65 days post ASAQ). Including the ALT liver 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 were 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 3**). 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 3: 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.

1.4.5 Mali (NIAID Protocol: 18-I-N084)

Healthy Malian adult 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 and 16

weeks or a condensed regimen of 1, 8, and 29 days (#16 in **Table 1**). The study started in June 2018 and the 3rd vaccination was administered in September 2018. Currently, the participants are nearing the end of the malaria follow up phase post primary vaccination series with a plan for a booster dose in June/July 2019.

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 2**, 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 sequalae.

Table 2: 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 total AEs in Arm)

Vaccine efficacy started immediately after the third vaccination. 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 3** below).

Table 3: 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

1.4.6 **Tanzania, BSPZV1 + BSPZV2**

When healthy, young males in Tanzania (clinical trial BSPZV1 conducted by the Ifakara Health Institute, (#5 in **Table 1**) 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.

In the BSPZV2 trial conducted in Bagamoyo, Tanzania (#8 in **Table 1**), 6/6 (100%) volunteers receiving 3 doses of $9x10^5$ PfSPZ of PfSPZ Vaccine (total dose $2.7x10^6$ PfSPZ) at 1, 9 and 17 weeks were protected against homologous CHMI conducted 3-10 weeks after last dose of vaccine, whereas all 6 controls became infected (Jongo, submitted). Interestingly, as might be predicted from the results in malaria-naïve volunteers, a 50% higher dose of vaccine administered on the same schedule protected only 33% of volunteers – thus, beyond a certain threshold, higher doses did not appear to be better, duplicating the results seen in malaria-naïve individuals.

1.4.7 **Germany, MAVACHE**

In a phase 1 dose escalation, regimen condensation, and dose number reduction study in healthy German adults, $9.0x10^5$ PfSPZ of PfSPZ Vaccine was administered by DVI on 1, 8, and 29 day schedule. This regimen was also found to be safe and tolerable and resulted in protection of 14/17 (78.8%) volunteers against 3- or 10-week homologous CHMI and 10/12 (83.3%) against 3- or 10-week heterologous CHMI.

1.4.8 **Mali (NIH Protocol 19-I-N113)**

MRTC/LMIV/Sanaria initiated a phase 2, randomized double blind, placebo-controlled study to assess the safety, tolerability, immunogenicity, and protective efficacy against *Pf* malaria infection of the 1, 8, 29 day PfSPZ Vaccine regimen given at a dose of $9x10^5$ PfSPZ (n=100) or $1.8x10^6$ PfSPZ (n=100) or placebo (normal saline; n=100) in healthy adult (18-38 year-old) WOCBP who anticipated becoming pregnant in the future. This design was selected to allow an assessment of PfSPZ Vaccine safety for pregnancy when the vaccine was administered prior to the pregnancy, to detect any safety signals that might be associated with the immune response to the vaccine.

The study started in June 2019 in Ouélessébougou and surrounding villages in Mali [#19-I-N113 (NIH) / Nº2019/52/CE/FMPOS (Mali)]. 324 female participants actively using contraception initially enrolled and underwent day -14 blood draw, of which 320 received artemether-lumefantrine (AL) prior to scheduled vaccinations #1 and #3. All study vaccinations were completed between 10 July to 27 August 2019.

Women who enrolled in this study reported planning to become pregnant in the next 1-2 years and were actively followed post vaccination for pregnancy. Those women who were found to be

pregnant during the first year after vaccination were followed for the course of their pregnancy, through delivery, and at least 1 year post-partum. Infants born to these women were also followed for 1 year post delivery. Follow-up in these participants is expected to be completed in April 2022

The study was extended for another malaria season (September 2020 to February 2021), without any additional study intervention, to explore the durability of vaccine efficacy. Women who became pregnant from this point further were only followed for the duration of the pregnancy and their offspring were followed only for delivery outcomes.

All participants and their offspring remained blinded for the entire duration of the study until the end of the second malaria season (~February/March 2021). All women and their associated offspring completed follow-up by April 2022.

Vaccinations were overall well tolerated by participants. The majority of AEs seen in every study arm were mild (Grade 1). There were no statistical differences seen between vaccine arms ($9x10^5$ PfSPZ Vaccine, $1.8x10^6$ PfSPZ Vaccine) and normal saline (placebo) for total AEs, related AEs, solicited AEs (local, systemic, individual AEs), laboratory abnormalities, nor vital sign changes.

Per protocol, participants were followed actively every two weeks (for 24 weeks post dose #3) and passively for unscheduled sick visits since receipt of Vaccination #3. After the completion of Year 1 malaria follow-up (24-week period post vaccination #3), protective efficacy analysis was completed on a group basis (see **Table 4** below) to determine if any significant protection was seen to rationalize continuing to follow the study population for another year, including another malaria transmission season.

Modified intention-to-treat (mITT) was the primary vaccine efficacy analysis and was based on *time to the first infection*, which was defined ≥ two Pf parasites per 0.5 milliliters (mL) by thick blood smear examination. The two vaccination arms were compared to placebo arm by logrank test for interval-censored data. Vaccine efficacy was defined as one minus the hazard ratio which was estimated from proportional hazard model accounting for interval censoring with baseline survival function as Weibull. VE analysis was completed by time to first infection (or time to event, TTE), binary (1-relative risk) as well as for the mITT and per protocol populations and are summarized below in **Table 4**.

In both PfSPZ Vaccine arms, in both populations (mITT and PP), there was statistically significant and consistent protective efficacy seen by both TTE and by proportional analysis (binary) (**Table 4**).

Table 4. Protective efficacy, by mITT and PP, against Pf Infection 24-weeks post dose 3 (Malaria Season Year 1, Aug 2019-Feb 2020)

	TTE (modified ITT)			TTE (per protocol)		
	VE (%)	95% CI	p value	**VE (%)**	95% CI	p value
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	**41%**	15, 59	0.007	**41%**	14, 59	0.008
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	**54%**	34, 69	<0.001	**57%**	36, 71	<0.001
	Binary (modified ITT)			**Binary (per protocol)**		
	No. infected/ No. per arm	**VE (95% CI)**	p value	No. infected/ No. per arm	**VE (95% CI)**	p value
Normal saline on 1, 8, 29 days	69/94			65/87		
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	52/94	**25% (5%, 41%)**	0.015	52/92	**24% (5%, 41%)**	0.016
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	47/100	**36% (18%, 51%)**	<0.001	44/95	**38% (20%, 53%)**	<0.001

Additional vaccine efficacy analysis was completed to look at clinical malaria, defined as the presence of asexual *P. falciparum* parasites at any parasitemia level and/or positive rapid diagnostic test 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. During the first malaria transmission season immediately post dose 3, there was protective efficacy seen in both PfSPZ Vaccine arms by mITT and PP analyses for TTE (46-48%) and the proportional (32-33%) endpoint (**Table 5**).

Table 5. Protective efficacy, by mITT and PP, against clinical malaria 24-weeks post dose 3 (Malaria Season Year 1, Aug 2019-Feb 2020)

	TTE (modified ITT)			TTE (per protocol)		
	VE (%)	95% CI	p value	**VE (%)**	95% CI	p value
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	**47%**	20, 65	0.004	**45%**	17, 64	0.007
PfSPZ Vaccine $18x10^5$ on 1, 8, 29 days	**48%**	22, 65	0.001	**48%**	21, 66	0.002
	Binary (modified ITT)			**Binary (per protocol)**		
	No. clin. malaria/ No. per arm	**VE (95% CI)**	p value	No. clin malaria/ No. per arm	**VE (95% CI)**	p value
Normal saline on 1, 8, 29 days	57/94			53/87		
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	38/94	**33% (9%, 52%)**	0.008	38/92	**32% (7%, 51%)**	0.013
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	40/100	**34% (10%, 52%)**	0.006	38/95	**34% (10%, 53%)**	0.007

Given the results reported above, the protocol was amended to follow women (pregnant and non-pregnant) for another year. No additional study product (vaccinations nor schedule anti-malarial drug) was provided prior to Year 2 follow-up, which started in September 2020.

As noted above, participants (pregnant and non-pregnant) were reconsented in September 2020 and followed in the same manner as in Year 1 with active visits every two weeks (for 24 weeks post reconsent) and passively for unscheduled sick visits. After the completion of Year 2 malaria follow-up (24-week period post reconsent = March 2021) protective efficacy analysis was completed using the same parameters as Year 1 (see **Table 6** below).

In both PfSPZ Vaccine arms, in the mITT, there was continued statistically significant and consistent protective efficacy seen by both TTE and by proportional analysis (binary) (**Table 6**). This continued to be seen in the $9x10^5$ PfSPZ Vaccine Arm for the PP population but was not statistically significant for the $1.8x10^6$ PfSPZ Vaccine Arm.

Table 6. Protective efficacy, by mITT and PP, against Pf Infection 24-weeks post Reconsent (Malaria Season Year 2, Sept 2020-Mar 2021)

TTE (modified ITT)				**TTE (per protocol)**		
	VE (%)	95% CI	P value	**VE (%)**	95% CI	p value
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	**61**	36, 77	0.001	**58**	28, 75	0.005
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	**45**	13, 65	0.029	**42**	6, 64	0.068
Binary (modified ITT)				**Binary (per protocol)**		
	No. infected/ No. per arm	VE (95% CI)	p value	No. infected/ No. per arm	VE (95% CI)	p value
Normal saline on 1, 8, 29 days	50/84			44/77		
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	28/84	**44% (19, 62)**	0.001	28/82	**40% (13, 60)**	0.006
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	38/92	**31% (5, 50)**	0.023	36/87	**28% (-2, 49)**	0.063

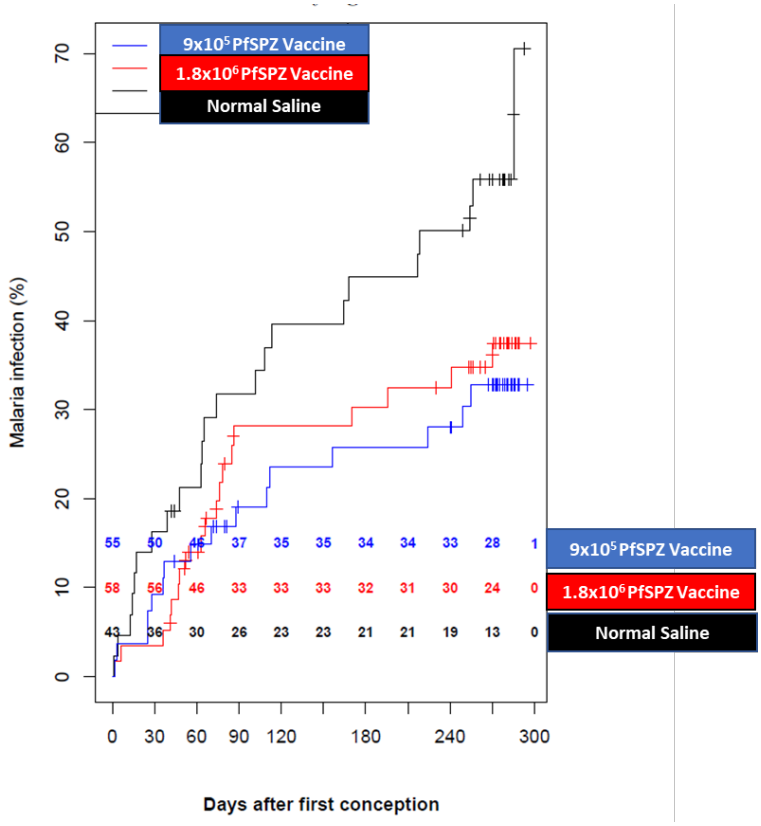
As in Year 1, clinical malaria was also explored as a malaria endpoint. As seen in **Table 7**, significant protective efficacy persisted for a second malaria transmission season for both PfSPZ Vaccine arms by TTE (45-50%) and proportional analysis (31-35%).

Table 7. Protective efficacy, by mITT and PP, against clinical malaria 24-weeks post Reconsent (Malaria Season Year 2, Sept 2020-Mar 2021)

TTE (modified ITT)				**TTE (per protocol)**		
	VE (%)	95% CI	P value	**VE (%)**	95% CI	p value
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	**56**	22, 75	0.008	**50**	12, 72	0.026
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	**40**	2, 64	0.069	**34**	-11, 61	0.158
Binary (modified ITT)				**Binary (per protocol)**		
	No. clin. malaria/ No. per arm	VE (95% CI)	p value	No. clin. malaria/ No. per arm	VE (95% CI)	p value
Normal saline on 1, 8, 29 days	39/84			34/77		
PfSPZ Vaccine $9x10^5$ on 1, 8, 29 days	23/84	**41% (9, 63)**	0.016	23/82	**36% (0, 61)**	0.051
PfSPZ Vaccine $1.8x10^6$ on 1, 8, 29 days	31/92	**27% (-7, 52)**	0.116	30/87	**22% (-18, 49)**	0.268

Exploring protective efficacy further in our pregnant population, we looked specifically at malaria-related events occurring during pregnancy. When analyzing Pf parasitemia during a woman's first pregnancy post vaccination (over a two year period), with accounting to timing of conception as a regressor, we saw continued protective efficacy through pregnancy by time to first infection for both PfSPZ Vaccine arms (see **Figure 4** for per protocol population; PfSPZ Vaccine $9x10^5$: VE 57%, CI 15-78%; p=0.0148; PfSPZ Vaccine $1.8x10^6$: VE 49%, CI 2-73%, p=0.0447) and proportional analysis (PfSPZ Vaccine $9x10^5$: VE 48%, CI 11-73%; p=0.0125; PfSPZ Vaccine $1.8x10^6$: VE 38%, CI 1-67%, p=0.0418). When completing the same analysis on the mITT population this finding continued to be seen in both PfSPZ Vaccine arms for both analyses with similar VE values but lost significant for time to first infection in the $1.8x10^6$ PfSPZ Vaccine arm (VE 46%, CI -3-72%, p=0.06).

Figure 4. Vaccine efficacy, by PP, against Pf Infection during first pregnancy over a two year period.



Given these promising results and in particular durability (over 2 years) of protection seen with PfSPZ Vaccine in non-pregnant and pregnant women, we would like to explore further if vaccine efficacy continues over another defined malaria season 3 years post recent primary vaccination to see if protection against parasitemia and clinical malaria continues.

1.5 Pregnancy Registry in Oulessebougou, Mali

Placental malaria (PM) leads to poor outcomes for pregnant women and their babies, and is caused by *Plasmodium falciparum* sequestration in the intervillous spaces of the placenta and ensuing inflammation. Severe maternal anemia, low birth weight (LBW) delivery and fetal loss are common sequelae. Malaria-related LBW alone is estimated to cause 62,000-363,000 infant deaths in Africa each year. To reduce poor pregnancy outcomes associated with malaria infection, WHO recommends monthly anti-malarial treatment with sulfadoxine pyrimethamine (SP) during the second and third trimester and the use of insecticide-treated bed nets. However, in East and Southern Africa, SP has lost its efficacy due to the spread of drug-resistant parasites. Therefore, an effective vaccine for PM is needed. Although the ideal vaccine will be given to adolescent girls prior to becoming pregnant, at this early stage of pregnancy malaria vaccine development, it is unknown if a boosting dose may be needed during pregnancy.

Before testing new interventions to improve pregnancy outcomes, it is important to obtain baseline information on pregnancy outcomes in the target population. In our recent longitudinal study (NIAID Protocol #10-I-N-156) of 1,885 pregnant women in Mali, in which women were

closely followed during pregnancy (including malaria prevention and treatment), 2.9%, 2.4% and 6% of pregnancies ended in miscarriage, stillbirth, and preterm delivery, respectively.

The primary goal of a larger follow-up study in Oulessebougou was put in place in 2017 (NIAID Protocol #17-I-N018) to assess pregnancy outcomes in women in the community with access to the WHO recommended standard of care. In the first part of the study, all women presenting for an antenatal visit at Ouelessebougou health centers were recruited into the study. In the second part of the study, non-pregnant women were enrolled and followed to capture data on pregnancy outcomes including early pregnancy losses. Pregnancy outcome information have been collected after the birth of the child or after pregnancy termination (i.e. miscarriage/stillbirth).

Under this protocol, between February 2017 and May 2018, ~1800 women consented to participate in the study (**Table 7a**). The proportions of miscarriage/stillbirths and early neonatal deaths was 4.3%, similar to that observed in our longitudinal cohort study, but women were often identified as pregnant starting in their second trimester of pregnancy.

Then, non-pregnant women of child-bearing age who were not under contraception or in early breastfeeding (<12 months) and were planning to become pregnant were recruited into the study and were followed prospectively in the second cohort starting in November 2018 and followed monthly to identify those who became pregnant. To date, as seen in **Table 7b**, the study has had ~800 enrolled WOCBP, of which 6% per month of the enrolled WOCBP have become pregnant with 10.4% of those detected early pregnancies during the first visit resulting in miscarriage thus far.

Overall pregnancy outcomes by age for the study, including women enrolling early in the first trimester as well as those enrolling late during their pregnancy, are summarized by age as noted below in **Table 7c**. Additional follow-up in non-pregnant women of child-bearing potential is ongoing and information regarding targeted outcomes will continue to help inform the community background rate of adverse events during pregnancy.

Table 7: Oulessebougou Pregnancy Registry (#17-I-N018)

Table 7a. Total Enrollment to Date by Age in Oulessebougou Pregnancy Registry (#17-I-N018)

Age group	N (%)
<18	228 (12.7%)
18-24	791 (43.9%)
25-30	499 (27.7%)
31-35	181 (10.0%)
36-40	93 (5.2%)
>=41	10 (0.6%)
Total	1802

Table 7b. Enrollment of WOCBP and Subsequent Pregnancies in Oulessebougou Pregnancy Registry (#17-I-N018)

Enrolled WOCBP	Number of Monthly Visits Completed	Number of Women Seen per Month	Number (%) of New Pregnancies	Total Number (%) of Pregnancies	Number (%) of Miscarriage
799					
	1	799	48 (6%)	48 (6%)	0 (0%)
	2	696	41 (6.3%)	84 (10.5%)	5 (10.4%)[1]

[1] % Miscarriage calculated based on the (number of reported miscarriages that month)/(number of detected and ongoing pregnancies the months prior) x 100

Table 7c. Pregnancy Outcomes by Age Group in Oulessebougou Pregnancy Registry (#17-I-N018)

Outcome	Age group	OR (95% CI)	p-value
Fetal loss	<18	2.7 (1.25-5.85)	0.01
	18-24	1.66 (0.86-3.19)	0.1
	25-30	REF	
	31-35	.74 (0.71-4.26)	0.2
	36-40	0.9 (0.2-4.07)	0.9
	>=41	9.12 (1.76-47.2)	0.008
Preterm Delivery	<18	6.13 (2.77-13.56)	<0.0001
	18-24	3.06 (1.48-6.36)	0.003
	25-30	REF	
	31-35	0.94 (0.25-3.52)	0.9
	36-40	5.20 (1.95-13.88)	0.001
	>=41	No cases	

2 Clinical Trial Rationale

2.1 Rationale for Accelerated PfSPZ Vaccine Schedule

There is ample evidence that the dose of PfSPZ Vaccine plays an important role in the development of protective immunity, but the importance of the dosing schedule is not as clear. A number of PfSPZ Vaccine studies have been completed and are currently ongoing to determine a highly effective vaccine schedule but given the targeted population in this study and subsequent proposed studies, a regimen that can be completed over a short period of time is ideal in order to quickly induce protection as early as possible in pregnancy.

Perhaps surprisingly to many, women generally do not receive any preventive measures during their first trimester, when most antimalarial drugs are contraindicated. Even in the presence of preventive measures such as IPTp, which entails periodic full antimalarial treatment after the first trimester, pregnancy malaria still occurs at a high rate. By inducing protection either prior to

pregnancy or during the first trimester of pregnancy, providing a longer window of protection may result in improved pregnancy and neonatal outcomes.

In addition, an accelerated approach could be deployed as a seasonal malaria immunoprotection for pregnant women living in the Sahel, similar to current seasonal malaria chemoprophylaxis (SMC) regimens used in children. In order for this to be implementable, a regimen that can be completed over a short period of time leading up to the seasonal malaria transmission season would be needed. Even if the protection seen with PfSPZ Vaccine is not long lived, in women who are pregnant, prolonged protection (years) is not required to see significant benefit to the mother and child.

In previously completed studies, a condensed PfSPZ Vaccine regimen of 1, 8, and 29 days has been found to be safe and well tolerated in multiple studies conducted in malaria naïve and malaria experienced individuals (as described in Section 1.4.2-1.4.7).

2.2 Rationale for PfSPZ Vaccine Doses of $1.8x10^6$ and $9x10^5$

The initial hypothesis underlying the sequence of studies completed and/or planned with PfSPZ Vaccine was that higher doses of PfSPZ Vaccine 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 vaccine efficacy.

Findings of multiple studies have shown that the threshold dose hypothesis is only partially true and is not as definitive as expected. The initial studies did confirm that vaccine efficacy increased with the dose of PfSPZ Vaccine administered and there was a clear dose response seen in several cases with increasing vaccine efficacy with increasing PfSPZ Vaccine doses (Epstein et al., 2017; Ishizuka et al., 2016) However, as individual doses were increased above about $9.0x10^5$ PfSPZ Vaccine in malaria naïve and experienced subjects, further improvements in vaccine efficacy were not consistently seen.

Given these preliminary results, in this study we propose to evaluate a known efficacious dose in an adult malaria exposed population ($1.8x10^6$ PfSPZ Vaccine) as well as the optimal dose proposed by the sponsor, Sanaria Inc. ($9.0x10^5$ PfSPZ Vaccine) to assess if dose amount given in the same dosing regimen (1, 8, 29 days) will provide increased protection in this population.

2.3 Rationale to Include WOCBP in Malaria Clinical Trials

Women of childbearing potential (WOCBP) are often excluded from participating in clinical trials owing to concerns about adverse maternal and fetal effects due to vaccination. But we believe it is important that vaccine developers consider the need for early transition of efficacious products to trials in young women; a generally underappreciated population in vaccine development.

2.4 Rationale to Develop a Vaccine to Prevent Malaria in Pregnant Women

Even in the presence of preventive measures such as IPTp, pregnancy malaria occurs frequently. Many women develop malaria before their first IPTp dose, which is only administered when

women present for their first antenatal visit, typically around 20 weeks gestation in many communities. Less than 20% of African mothers receive the recommended 3 doses of IPTp during pregnancy. Other recommended interventions such as insecticide-treated bed nets and case management of intercurrent malaria infections are variably implemented, but even in areas of relatively good uptake the rate of pregnancy malaria remains high. Further, drug-resistant parasites and insecticide-resistant mosquitoes are inexorably spreading, which will further erode the benefits of existing interventions. Thus, a vaccine to specifically reduce pregnancy malaria or a universal vaccine to reduce all malaria, is urgently needed to improve pregnancy outcomes. To have maximum benefit, an effective vaccine should be administered before first pregnancies but also needs to be assessed if can be given safely during pregnancy as a primary series and/or booster.

Specific vaccines to prevent malaria in pregnant women could potentially be an important method of preventing malaria in pregnancy but their rationale, and the current approaches to research, development and clinical evaluation require further consideration. Any successful vaccine aimed at preventing malaria infection or disease would be expected to reduce the burden of malaria in pregnant women. A vaccine with lifelong efficacy could be given with other childhood vaccines but a vaccine with shorter duration of efficacy could potentially be delivered at a similar time as a vaccine for prevention of HPV, providing boosting prior to pregnancy, or be given early during pregnancy.

No reproductive toxicity studies have been performed yet on PfSPZ Vaccine to assess preclinical safety and immunogenicity in pregnant animals, but is planned to occur in 2019. These studies are planned to assess inoculation at different stages of pregnancy and investigate whether PfSPZ can cross the placenta and/or cause adverse outcomes in pregnancy and/or to the fetus. Once Reproductive toxicity studies have been completed and reviewed and safety data has been reviewed from the first year of this study, as noted prior, our clinical development plan for PfSPZ Vaccine in WOCBP and pregnant women, would next include studies of the safety and efficacy of a primary immunization series and studies to evaluate the efficacy of boosting during pregnancy.

2.5 **Rationale for Assessing PfSPZ Vaccine in WOCBP**

Malaria is a scourge of pregnant women in developing countries, causing maternal, perinatal, and infant mortality. Current tools (drugs, bed nets) are losing activity and the search for new safe tools has been unsuccessful. Further, as noted prior, no malaria vaccine has ever been tested in WOCBP or pregnant women in Africa, and no vaccine of any kind is FDA-approved to date for use in pregnancy, highlighting the paucity of data in a highly malaria-impacted population.

When tested in healthy, non-pregnant, non-lactating adults, and in children and infants, Sanaria's vaccines are safe and well-tolerated, and protective in both malaria-naive and malaria-experienced adults. Sanaria has now amassed sufficient safety and protection data to justify studies in more vulnerable populations, such as HIV-infected individuals (study started in Tanzania in February 2018) and now in WOCBP and pregnant women. There are extensive data

from 3,652 immunizations into 1,130 individuals that PfSPZ Vaccine is safe, well tolerated, and able to protect against Pf infection for sustained periods.

In addition, PfSPZ Vaccine is formulated without an adjuvant thereby avoiding the systemic reactogenicity and safety concerns of more inflammatory products that may result in systemic inflammation resulting in poor pregnancy outcomes.

NIH/NIAID/LMIV, MRTC, and Sanaria hosted a two-day meeting of experts to develop a pathway to test the safety of the vaccines in pregnancy, to explore the need for malaria vaccines in pregnancy, and to conceptualize clinical trials to measure protective efficacy of PfSPZ-based vaccines against malaria infection during pregnancy. A main conclusion of the meeting was that the potential benefits of PfSPZ immunization greatly outweigh hypothetical risks during pregnancy, and therefore there is an ethical imperative to proceed with testing.

Our vision is for PfSPZ Vaccine to be scaled up as a public health tool that prevents malaria in WOCBP and pregnant women. We have proposed to evaluate this vaccine first in WOCBP with booster doses being evaluated post receipt of primary series, especially at the onset of pregnancy. Rolling out studies in a controlled stagger manner, we would like to then move to giving pregnant women, who have not been immunized prior, a primary series during various trimesters of pregnancy.

In addition, as already planned in Equatorial Guinea, mass vaccination programs will need to include women of reproductive age and pregnant women to achieve high vaccine coverage. To achieve the requisite high population coverage, the vaccine must be safe in all age and risk groups, including women of reproductive age and pregnant women. In addition to the direct protection that PfSPZ Vaccine might offer pregnant women, the plan for mass vaccination programs, mandates that we establish the safety, tolerability and efficacy of PfSPZ-based vaccines in pregnancy.

2.6 Rationale for Following WOCBP and Pregnant Cohort for Another Malaria Transmission Season

Limited data are available on the protective efficacy durability and longevity of PfSPZ Vaccine over two independent malaria transmission seasons. Combined evaluation of PfSPZ Vaccine VE over two malaria transmission seasons was assessed for a PfSPZ Vaccine study in Burkina Faso (NCT02663700; unpublished) which did show significant efficacy over two years combined by time-to-event analysis. To confirm this finding, we would like to continue to follow our study cohort in a blinded fashion for a second transmission season. We will examine vaccine efficacy during the 24 week period encompassing the second transmission season, and also over the entire two year period after vaccination. Given continued protective efficacy seen in non-pregnant and pregnant women during the second transmission season, we will examine vaccine efficacy during another 24 week period encompassing a malaria transmission season three years post initial vaccinations.

The question of longevity is key for vaccine development in WOCBP and pregnant women and future clinical trial designs for PfSPZ Vaccine in this population; in particular addressing

questions regarding timing and need for booster vaccinations. Knowing how long a primary PfSPZ Vaccine series sustains protection, without additional intervention, will address important unknowns that will be essential to answer.

3 STUDY OBJECTIVES

3.1 Primary Objective

Safety: Assess safety and tolerability of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses (9.0×10^5 and 1.8×10^6)

3.2 Secondary Objective

Protective Efficacy: Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses ($9.0 \times 10^5$and 1.8×10^6)

3.3 Exploratory Objectives

Protective Efficacy:

- Explore the impact of PfSPZ Vaccine on additional malaria infection endpoints

Immunogenicity:

- Characterize and compare host immune responses to malarial antigens and host proteomic profiles and transcriptomes in African WOCBP 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
- Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses ($9 \times 10^5$and 1.8×10^6) for a second malaria transmission season
- Assess the efficacy of PfSPZ Vaccine primary series in healthy Malian WOCBP when given at 1, 8, 29 days at two doses ($9 \times 10^5$and 1.8×10^6) for another malaria transmission season (durability)

Pregnant Women and Neonates

Safety:

- Assess maternal and neonatal outcomes post receipt of PfSPZ Vaccine primary series

Protective Efficacy:

- Explore the PfSPZ Vaccine protective efficacy and durability of protection in pregnant women.

- Explore the PfSPZ Vaccine protective efficacy and durability of protection in neonates/infants born to PfSPZ vaccinated mothers.

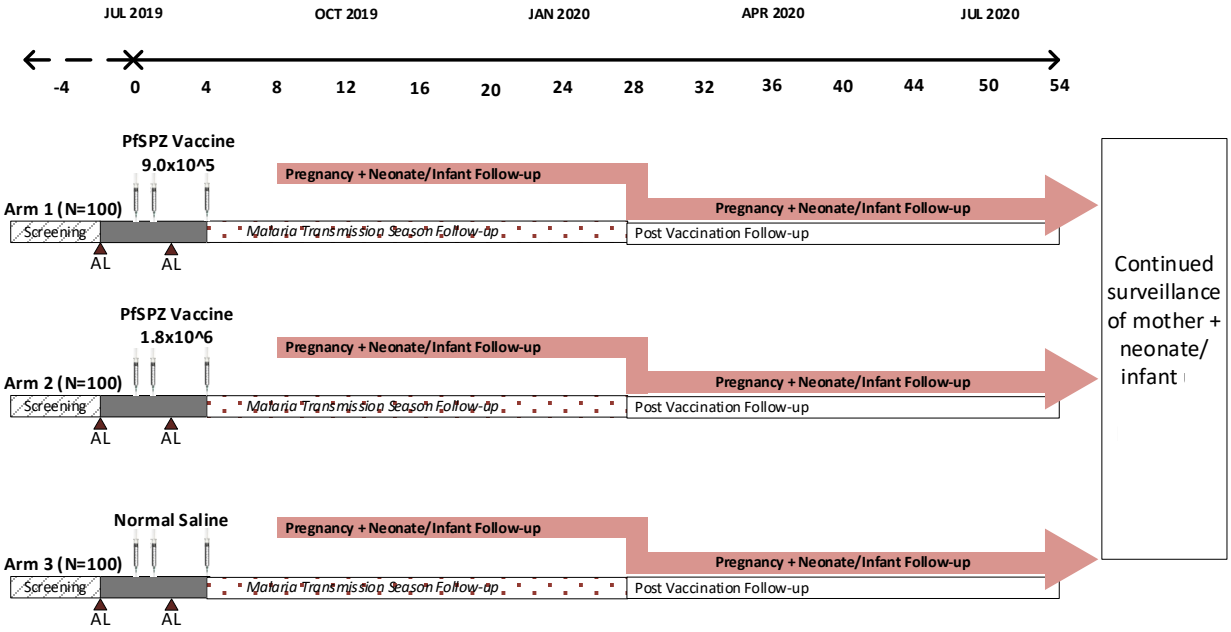
4 STUDY DESIGN

This study will enroll healthy Malian adult WOCBP between 18 and 38 years of age to participate in a randomized, double blind, placebo-controlled study to assess the safety, immunogenicity and protective efficacy of PfSPZ Vaccine across a year of follow up including malaria transmission season. Participants will be immunized with a 3-dose series of $9.0x10^5$ PfSPZ Vaccine or 3-dose series of $1.8x10^6$ PfSPZ Vaccine or normal saline (placebo) at 1, 8, 29 days (as seen below in **Figure 5).** Vaccinated subjects and controls will then be assessed for malaria infection during the ensuing malaria transmission season and subsequent dry season, including following prospectively women who become pregnant during follow up and their offspring. Volunteers will be randomized into three arms at a 1:1:1 ratio to receive PfSPZ Vaccine versus normal saline control.

Approximately one year post receipt of vaccination #3 (2019), women, both pregnant and non-pregnant, will be asked to continue on study for at least another 6 months. No additional study products will be given during this time. If a woman becomes pregnant during this extended follow-up, she will continue to be followed through the course of her pregnancy and shortly after delivery. Subsequent offspring born from pregnancies from this time period will only be followed for delivery outcomes.

Approximately three years post receipt of last vaccine dose, women, both pregnant and non-pregnant, will be asked to be followed for another 6 month period. No additional study products will be given during this time. If a woman is or becomes pregnant during this extended follow-up, she will continue to be followed during the 24 week period, but not beyond that follow-up period. Subsequent offspring born from pregnancies from this time period will not be followed.

Figure 5: Study Schema (Year 1)



4.1 Study Arms

Three study arms will be enrolled in this study, with two PfSPZ vaccination arms and one control arm.

Arm 1: (n= 100) will receive 3 doses of PfSPZ Vaccine (9.0×10^5) via direct venous inoculation (DVI) at 1, 8, 29 days.

Arm 2: (n= 100) will receive 3 doses of PfSPZ Vaccine (1.8×10^6) via DVI at 1, 8, 29 days.

Arm 3: (n=100): will receive 3 doses of placebo saline injection via DVI at 1, 8, 29 days.

All injections will be administered by DVI. All volunteers will receive antimalarial treatment with artemether/lumefantrine (AL) approximately two weeks prior to the 1st and 3rd injection. Post 3rd injection, participants will be followed through the malaria transmission (rainy) season, approximately 6 months, and then the ensuing dry season for an additional 6 months. Participants will be monitored for safety, immunogenicity, and protective efficacy (malaria infection) during the follow-up period.

For any women who becomes pregnant during the first year of the trial, follow up will continue through the end of pregnancy, and any viable newborns/infants and their mothers will be followed through the first year of life.

During the second year of follow-up, which will start immediately at the conclusion of the first year of follow-up, participants will be followed through the malaria transmission (rainy) season,

approximately 6 months. Participants will be monitored for safety (unsolicited AEs), immunogenicity, and protective efficacy (malaria infection) during the follow-up period.

For any women who becomes pregnant during the second year of the trial (~September 2020 to March 2021), follow up will continue through the end of pregnancy and through ~1-2 months post delivery, but any viable newborns/infants will not be followed beyond delivery.

Given continued protective efficacy seen during Malaria Season Year 2 (2020-2021), women (pregnant or non-pregnant) who have received at least two vaccinations will be asked to come back to be followed for a 24-week period during the 2022-2023 Malaria Season. No product (AL nor PfSPZ Vaccine nor Normal Saline) will be administered.

5 STUDY ENDPOINTS

5.1 Primary Endpoints

Safety: Incidence of local and systemic adverse events (AEs) graded by severity occurring within 7 days after each vaccine administration.

5.2 Secondary Endpoints

Protective Efficacy: *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 over 24 weeks (all Arms).

5.3 Exploratory Endpoints

Protective Efficacy

- Measurement and comparison of various malaria infection endpoints, such as PCR, gametocytemia, non-falciparum parasitemia, symptomatic malaria
- *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 approximately 1 year post receipt of 3rd injection for 24 weeks.
- *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 approximately 3 years' post receipt of last injection for 24 weeks.

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.

Pregnant Women and Neonates

Safety:

- Incidence of maternal obstetric outcomes (miscarriage/stillbirth, intrauterine growth restriction, hypertensive diseases in pregnancy, preterm delivery) and neonatal outcomes (neonatal death, low birth weight, small for gestational age, major malformations, and microcephaly)

Protective Efficacy:

- *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) during the pregnancy, during infancy, and starting immediately following 3rd injection
- *P. falciparum* placental infection defined as any positive placental blood smear for *P. falciparum*.

5.4 Sample Size and Estimated Duration of the Study

A total of 300 volunteers will be enrolled/randomized in this trial, 200 subjects in the vaccine arms (Arm 1, Arm 2) and 100 subjects in the control arm (Arm 3). WOCBP will be enrolled in the study for approximately 21 months if they do not become pregnant during the course of the study.

If a woman becomes pregnant during the course of the first year of the study, they will not be removed from the study, but at the time of pregnancy determination will no longer receive investigational product, but will be followed throughout the course of their pregnancy and post-delivery as outlined in **Appendix A.** Their offspring will also be followed as outlined in **Appendix A** for one year following birth.

If a woman becomes pregnant, but pregnancy does not result in a live birth during the study, she will not be removed from the study as long as she agrees to continue on the study as a non-pregnant participant. If a woman delivers and completes her post-delivery follow-up before the end of the second year of follow-up (~March 2021) she can be followed as a non-pregnant subject as long as she agrees to continue on the study.

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 before the administration of vaccine #1, then an updated medical review and laboratory testing (CBC with differential, ALT, Creatinine, ECG, Pregnancy test) + repeat malaria comprehension will be completed to determine eligibility for enrollment.

- **Eligible** – subjects will be considered eligible to enroll once they have completed the screening procedures and have **met all inclusion** criteria and **not met exclusion** criteria. They will be asked to provide baseline blood samples for the immunological assessment before the vaccination.
- **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 is determined eligible for enrollment after study completes enrollment.
- **Enrolled** – subject will be considered enrolled if they:
    - Meet eligibility criteria,
    - Undergo first blood draw on study day -14
- **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.
    - If a subject is discontinued/withdrawn and unscheduled, intentional unblinding is not determined to be needed at the time of discontinuation/withdrawn she will be contacted on study day 561 for notification of her arm assignment (individual unblinding).
- **Completed** – subjects are considered completed when they complete the final study visit.
    - Never pregnant women = study day 561
    - Pregnant women = study day P16 (~12 months following delivery) for Year 1; study day P11 (~2 months follow delivery) for Year 2
        - If pregnancy does not result in a live birth, will rejoin the non-pregnant follow-up schedule until either 1) Pregnant; 2) End of Study (study day 561) for non-pregnant women
    - Offspring of WOCBP = study day C6 (~12 months of age) for Year 1 pregnancies only
    - Year 2 offspring will only have delivery outcomes (study day C0) collected

6 STUDY AGENTS

6.1 **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] 21), 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.

6.1.1 **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.

6.1.2 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.

6.1.3 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.

6.2 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.

6.2.1 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.

6.2.2 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.

6.2.3 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.

6.2.4 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.

6.3 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.

6.3.1 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).

6.3.2 Disposition and Dispensation

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

6.3.3 Administration and Dosage

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

6.3.4 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.

6.4 Antimalarial Drugs

All antimalarial medications used for the study will be maintained at the study site and administered by direct observational therapy. Artemether/lumefantrine (AL) 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.

6.4.1 Preparation and Administration

AL will be provided as tablets for oral administration. Administration will be under direct observation by study staff according to dosing parameters; on a case by case basis a dose may be given outside of directly observed therapy (DOT; e.g. during nighttime dosing) as long as timing of dose and overall compliance can be confirmed.

6.4.2 Storage and Handling

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

6.4.3 Return of Study Product

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

6.4.4 Drugs used for Pre-emptive Treatment of Malaria

During this study, all Arms will receive pre-emptive antimalarial treatment prior to 3rd injection.

AL (e.g, 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.

6.4.5 Drugs Used for Management of Symptomatic Malaria

In accordance with the Malian Government treatment guidelines, volunteers will be treated with AL (e.g. Coartem®, 80mg/480mg per dose or equivalent). AL 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 and/or positive rapid diagnostic test 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.

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

This study will not interfere with the routine standard-of-care. Pregnant women will receive medical care through the antenatal clinic (ANC) according to the standard of care in Mali. The study will support the public health clinic by providing pregnancy testing, pregnancy dating by ultrasound, ensuring supply of RDT and blood smears for malaria diagnosis, and drugs for malaria treatment and prevention, and training on accurate completion of antenatal cards. During the study visits, the team will encourage women who became pregnant to attend ANC. The research conducted in this study will not influence medical advice given to subjects and individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy.

Neonates and infants will also be followed and treated for malaria in accordance with the Malian treatment guidelines. Uncomplicated malaria and asymptomatic parasitemia is treated with AL and complicated malaria is treated with arthemeter, artesune or quinine injection.

6.5 Contraindications to Vaccination

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

- 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 study for safety evaluation and continued efficacy analysis (blood smears).

For women who become pregnant during the course of the study, vaccination will be deferred at this time, but the women and their offspring will be followed according to **Appendix A.**

6.5.1 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), and if subject is willing longer, unless the subject has withdrawn consent. If the subject does not receive any further vaccinations, scheduled safety blood draws and malaria blood smear may not be performed at the discretion of the PI. Blood draws scheduled to measure immune response may be obtained if possible.

If a subject misses vaccination #2, but is eligible for vaccination #3, they can proceed with receipt of vaccination #3 and be followed per protocol.

6.6 **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 ≥20 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.

7 STUDY POPULATION

The study population will consist of healthy adult females of child bearing potential aged 18–38 years who reside in Ouelessebougou and surrounding villages in Mali.

7.1 Clinical Site

The study will be carried out in collaboration between the LMIV/NIAID/NIH and 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.

Ouelessebougou is located about 80 km south of Bamako, the capital city of Mali. It contains the district health center and a Clinical Research Center located in the community health center where studies of malaria and other infectious disease have been ongoing since 2008.

Based on our previous immunoepidemiology study in Ouelessebougou, malaria burden during pregnancy is high. Overall, at enrollment 28% of the pregnant women were infected with malaria, and infection was more frequent in primigravid women (43.5% compared to 19.8% of multigravid women). Among children less than 5 years of age, the incidence rate of clinical malaria was 1.99 episodes/child/year. Based on population census conducted under NIAID #17-I-N018, 23.9% (7061/29,569) are women of child bearing age (aged 15-45 years), and 14% of these women (988) were pregnant.

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, 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).

7.2 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 15.2.1**). 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.

7.3 Inclusion Criteria

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

1. Females of child bearing potential aged ≥ 18 and ≤ 38 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. Must be willing to use reliable contraception (defined as: pharmacologic contraceptives [parental delivery] or pre-existing intrauterine or implantable device) from 21 days prior to study day 1 to 28 days after last vaccination
7. Report being interested in becoming pregnant within the next 1 year

7.4 Rescreening Inclusion Criteria

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

1. Females previously enrolled and received at least 2 vaccinations and followed both during 2019-2020 malaria transmission season AND 2020-2021 malaria transmission season
2. Willing to have blood samples stored for future research

7.5 Exclusion Criteria

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

1. Pregnancy at the time of enrollment/vaccination, as determined by a positive urine or serum human chorionic gonadotropin (β-hCG) test
2. Biologically unable to become pregnant secondary to: surgical sterilization, premature ovarian insufficiency (defined as no menses for ≥12 months without an alternative medical cause)

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 (Hgb), WBC, absolute neutrophils, and platelets outside the local laboratory-defined limits of normal and ≥ Grade 2 (subjects may be included at the investigator's discretion for 'not clinically significant' abnormal values)

5. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal and ≥ Grade 2 (subjects may be included at the investigator's discretion for 'not clinically significant' abnormal values)

6. Infected with human immunodeficiency virus (HIV)

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.

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 2 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

17. Known asplenia or functional asplenia

18. Use of chronic (≥14 days) oral or IV corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone ≥20 mg/day) or immunosuppressive drugs within 30 days of vaccination

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

22. Known allergies or other contraindications against use of artemeter/lumefantrine

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

7.6 Rescreening Exclusion Criteria

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

1. Enrollment in another malaria vaccine (outside of this protocol) or drug trial in the last 2 years

2. Development of 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

7.7 Justification for the Exclusion of Pregnant Women

This study will not enroll pregnant woman at the start of the study since the effects of PfSPZ Vaccine on the developing human fetus will still be unknown at that time. No reproductive toxicity studies have been performed yet on PfSPZ Vaccine to assess preclinical safety and immunogenicity in pregnant animals, but is planned to occur in 2019. Once reproductive toxicity studies have been completed and reviewed and safety data has been reviewed from the first year of this study, as noted prior, our clinical development plan for PfSPZ Vaccine would include next studies of the safety and efficacy of a primary immunization series in all trimesters, and studies to evaluate the efficacy of boosting during pregnancy.

If a woman becomes pregnant after enrollment, she may continue in the study for follow up only as outlined in **Appendix A**. AL is considered a category C medication, though AL is recommended as first line therapy in Mali and the U.S. for women with malaria in the second and third trimester of pregnancy. There are no adequate studies in pregnant women during the first trimester.

For prolonged follow-up (2020-2021, 2022-2023) pregnant women are able to participate in the study and are not excluded from participation. During this period of the study, no study product (vaccine, anti-malarial drug) will be administered.

7.8 Justification for Exclusion of Children

Children are excluded from enrolling during the vaccination phase of this study given the focus of enrollment is on women who are looking to become pregnant in the next 1-2 years' time.

Children will subsequently be enrolled, if consented by their parents, at birth until 12 months' of age.

8 SCHEDULE

8.1 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 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 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 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 vaccination:

- Explain the study and informed consent document to the subject; reconsenting if on the same prior version of the ICF is not required.
- 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 15.2.2**) on the Malaria Comprehension Exam.
- Elicit a complete medical history, including menstrual and contraceptive history for females, and medication use.
- Confirm that females of childbearing potential are willing to use reliable contraception from at least the period from screening (at least 21 days prior to first vaccination) through 28 days after the third 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 venous blood for complete blood count (CBC) with differential and platelet count, ALT, creatinine (Cr), HIV testing

- Obtain urine for urinalysis and/or urine dipstick
- Obtain urine (or serum) for pregnancy testing.
- Obtain a 12-lead ECG.

Sickle cell testing will be completed retrospectively on subjects enrolled into the study. 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.

For continuation into Year 2 of follow-up, given no study product is being administered, women will be continued on study and no additional rescreening procedures, or inclusion/exclusion criteria will need to be completed.

8.2 **Rescreening**

The purpose of the rescreening visit is to determine subject eligibility for study participation. Screening procedures include the informed consent process, review of eligibility, and a focused physical examination, if clinically indicated, to restart another 24 week period of follow-up (including blood draws). No screening labs, imaging, or ECG will be required given no additional study product is being administered.

8.3 **Assignment of Groups**

Randomization of the subjects in Arm 1 or Arm 2 or Arm 3 will occur prior to first vaccination. Once a subject has received their first vaccination, they 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), 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.

8.4 Detailed Study Procedures

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

9 STUDY PROCEDURES/EVALUATIONS

9.1 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.

9.2 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 (Hgb), and platelet count.
2. Serum creatinine (Cr)
3. Alanine aminotransferase (ALT)
4. HIV test (can include rapid diagnostics, ELISA, Western Blot if indicated) (at screening only)
5. 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)

Additional clinical testing may be completed if a woman becomes pregnant (**Appendix A**):

1. Blood type
2. Serum glucose
3. Serum Cr and ALT
4. Urinalysis or urine dipstick
5. CBC with differential
6. Prenatal Ultrasound
7. Rubella serology
8. Hepatitis B serology
9. Syphilis by treponemal or non-treponemal testing
10. Toxoplasma IgG, IgM
11. Malaria blood smear or rapid diagnostic test (RDT)
12. Placental sample for parasitemia (blood smear, PCR) + histology

During pregnancy, some of the above safety clinical testing (see **Appendix A**) may be completed at least once per trimester at the women's OB/GYN provider or during a clinical visit. In addition, specified research labs in **Appendix A** during pregnancy may also be completed at least once per trimester.

Additional clinical testing may be completed in neonates/infants upon follow-up (**Appendix A**):

1. Hemoglobin
2. Malaria blood smear or RDT

9.3 Electrocardiogram

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

9.4 Malaria Diagnostics

9.4.1 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 smears are prepared in duplicate according to standard malaria challenge procedures and evaluated by trained study microscopists, and the results reported to the study PI. At least 0.5 µL are scanned for the presence of malaria parasites. This method allows for detection of a parasite density of approximately 3 parasites/µL and early diagnosis, often before subjects become symptomatic for malaria. For symptomatic subjects, at least 1.5 µL are evaluated. Slides are considered positive if at least two unambiguous *P.falciparum* parasites per slide are identified and confirmed by a second microscopist.

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.

9.4.2 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.

9.4.3 Placental Malaria

Blood smear (BS) microscopy, which is viewed as the gold standard for diagnosing placental malaria owing to longstanding clinical practice. Placental malaria will be detected by microscopy of Giemsa-stained thick and thin smears of blood extracted from placental tissue by mechanical grinding and will be quantified as percent of infected erythrocytes. Two readers will confirm each read.

Whether applied to peripheral blood or placental blood samples, PCR methods yield positivity rates 20% or more above those of BS microscopy. For placental samples, qPCR will be used retrospectively using similar methods as outlined in **Section 9.4.2**.

9.5 Unscheduled Blood Smear Positive Visits

After receipt of at least one vaccination, if a subject has detected *P falciparum* malaria parasites on peripheral thick blood smear (whether or not they were symptomatic; scheduled or unscheduled visits), she will be asked to the clinic to provide an additional blood sample within 48 hours 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 with the positive blood smear
- 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 4mL EDTA sample has been collected in the last 28 days for this purpose, it can be deferred at the investigator's or subject's discretion.
- 0.5 mL for qPCR if sample not obtained within the last two days with the positive blood smear

The unscheduled blood smear positive blood draw does not need to be completed with every positive blood smear and subjects have the right, as with every blood draw, to refuse to return for this additional blood draw. Preference for unscheduled blood smear positive blood draws is for first positive blood smear post vaccination #3, subsequent positive blood smear after treatment

with anti-malarial treatment (>14 days after treatment), and new positive blood smear after having a negative blood smear >28 days.

9.6 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*.

VAR2CSA is a member of the PfEMP1 variant antigens that is preferentially expressed by placental parasites, and is currently the leading candidate for a vaccine to prevent malaria during pregnancy. During pregnancy parasites sequester in the placenta with and without parasites detected in peripheral blood. Increased antibody levels to VAR2CSA can indicate exposure to placental infection. Antibody levels to VAR2CSA will be compared between women who will become pregnant and received PfSPZ vaccine or placebo.

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. Binding ELISA for antibodies to VAR2CSA in samples from women that will become pregnant during the study
3. 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)

9.7 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.

9.8 Maternal and Neonatal Clinical Outcomes

Pregnancy and neonatal outcomes will include the collection of the following information

1. Date of delivery, or date of the end of pregnancy in case of miscarriage/stillbirth
2. Miscarriage
3. Stillbirth

Macerated: fetus shows skin and soft-tissue changes (skin discoloration or darkening, redness, peeling and breakdown) suggesting death was well before delivery.

Fresh: Fetus lacks skin changes and is presumed to have died intrapartum birth.

4. Preterm delivery (based on ultrasound examination at recruitment at antenatal clinic, or recorded in the antenatal clinic card) or womb fundal height or last menstrual period.

Defined as gestational age <37 weeks.

5. Hypertensive disease in pregnancy (including gestational hypertension, preeclampsia, eclampsia, chronic hypertension with superimposed preeclampsia and HELLP (Hemolysis, Elevated Liver Enzymes, Low Platelets) syndrome)

6. Birth weight, length, and head circumference (recorded in the antenatal clinic card within 24 hours of birth; evaluation for intrauterine growth restriction, microcephaly).

7. Neonatal death

Early neonatal death: 0-7 days post delivery

Late neonatal death: 8-28 days post delivery

8. Major malformations

Includes: central nervous system abnormalities, cleft lip/palate, congenital heart defects, gastrointestinal abnormalities, limb abnormalities, reproductive system abnormalities.

Post-delivery questionnaire will provide the following information: maternal gravidity, history of pregnancy loss and socio-economic status, covariates that can influence the outcome of the current pregnancy.

Following delivery in Year 1, mothers will be periodically followed as outlined in **Appendix A**. If women become pregnant during Year 2 follow-up, they will be followed as outline in **Appendix A** through visit P11.

Infant follow-up, if pregnancy detected during Year 1 follow-up, will include regular measurement of weight, length, and head circumference as well as periodic blood draws as outlined in **Appendix A**. At 6- and 12-months of age, a developmental screening questionnaire will be completed. Infants born to women who become pregnant in Year 2 will only be followed for delivery outcomes.

If a woman is pregnant or becomes pregnant during 2022-2023 follow-up period (3 years post initial vaccination series), she will be followed similar to the non-pregnant population. If she delivers while on study (during the 24-week period) we will collect limited information about the delivery outcome (estimated weeks of gestation (or full term versus preterm) and major complications or pregnancy outcomes) but we will not collect blood, placental, or cord blood samples and we will not follow the infants. During follow-up in both the pregnant and non-pregnant population we will only report malaria and venipuncture related AEs and all SAEs.

10 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 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 NIHIRB.

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 NIH 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.

11 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 (**Section 15.3**). 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.

12 ASSESSMENT OF SAFETY

12.1 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.

12.2 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.

Routine hospitalizations (mother and infant(s)) related to normal delivery and post partum care will not be reported as SAEs unless they meet an additional SAE criteria for reporting. Duration of hospitalizations and type of delivery (e.g. vaginal or C-section) will be routinely noted in the delivery reports.

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.

Serious Unanticipated Problem (UP): A UP that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

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 research protocol.

> **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.

Non-Compliance: 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. Failure of subjects to comply with the research protocol does not represent non-compliance unless that failure is due to an action or omission of a member of the research team, for example, the failure to give adequate instruction to the subject

> **Serious non-compliance:** Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially effects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
>
> **Continuing non-compliance:** A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

12.3 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 8** 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 8: 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 B**, will be collected and graded for severity through 7 days after each vaccination until resolved.

For follow-up completed in 2022-2023 (3-years post receipt of last study product) only malaria related AEs (e.g. clinical malaria, blood smear positive) and venipuncture AEs and all SAEs will be captured.

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.

12.3.1 **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 9.**

Table 9: 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

12.3.2 **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.

12.4 Investigator Reporting Responsibilities to the Sponsor

12.4.1 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.

12.4.2 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. L.W. Preston Church, M.D.
Tel: 240-403-2714 (office)
Tel: 843-814-0888 (mobile)
Email: lwpchurch@sanaria.com

4. 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:
PI: Patrick Duffy, M.D.
Tel: 301-761-5089
Fax: 301-480-1962
Email: patrick.duffy@nih.gov

Independent Safety Monitor:
Oumar Moussokoro Traore, MD
Reference Health Center of Commune V
Bamako, Mali
+ 223 76 18 72 71
Email: baroutraore@yahoo.fr

12.4.3 **Unanticipated Problems (UPs)**

All UPs that are also adverse events will be reported to the IND sponsor on the NIH Reportable Events 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.

12.4.4 **Pregnancy**

Pregnancies are anticipated to occur during follow up of this population of WOCBP, and our ongoing pregnancy registry data suggest this may occur at a rate of ~6% per month in the target demographic once active pregnancy prevention stops. Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs; despite these events being reported as AEs or SAEs, based on our initial pregnancy registry data, we expect these events to occur at a relative frequency as noted below (**Table 10** and **11**).

Table 10: Rate of Adverse Outcomes from Pregnancy Registry (NIAID Protocol #17-I-N018)

	Primigravid, % (n)	Secundigravid, % (n)	Multigravid, % (n)
Miscarriage + stillbirth	2.2% (8)	2.2% (7)	2.0% (16)
Preterm delivery	7.5% (27)	4.1% (13)	2.6% (21)

Table 11: Rate of Early and Late Neonatal Death from Pregnancy Registry (NIAID Protocol #17-I-N018)

	Preterm Delivery, % (proportion)	Term Delivery, % (proportion)
Early neonatal death	11% (7/61)	1.6% (23/1404)
Late neonatal death	1.6% (1/61)	0.3% (4/1404)

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 during vaccination. At >1 month post third (or last) vaccination, given the number of expected pregnancies on this study, all pregnancies will be reported monthly to the IND sponsor and LMIV via fax or email with quarterly detailed updates as noted below.

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 via fax or email. At >9 months post third (or last) vaccination, given the number of expected deliveries on this study, all births will be reported monthly to the IND sponsor and LMIV via fax or email with quarterly detailed updates as noted below.

In the event of pregnancy during the period of vaccine administration (including 21 days prior to vaccination #1 to 1 month post vaccination #3), the following steps will be taken:

- The subject will be withdrawn from receiving any further investigational products but will continued to be followed actively in the study per **Appendix A;** alternative pregnant woman schedule but continued blood smear evaluation and safety lab evaluations per vaccine cohort (**Appendix A**)
- Report to FMPOS EC as an informational item
- Report to NIH IRB at the time of continuing review
- Report to DSMB, Sponsor Medical Monitor, and Site Medical Monitor
- Advise research subject to notify the obstetrician of possible study vaccine exposure

In the event of pregnancy or delivery after the period of vaccine administration (starting >1 month post dose #3 for pregnancies and >9 months post dose #3 for deliveries/miscarriages) and follow up, the following steps will be taken:

- The subject will continue in follow-up according to the alternative study schedule outlined in **Appendix A.**
- Reported to FMPOS EC and NIH IRB at time of continuing review.
- Report to DSMB, Sponsor Medical Monitor, and Site Medical Monitor quarterly.
- Advise research subject to notify the obstetrician of possible study vaccine exposure

Given women reconsenting to participate in an additional year of follow-up (2022-2023) may be pregnant to participate and it has been >3 years since last product administration, documentation of new pregnancies and/or birth outcomes will not be reported and will just be captured on the study CRF.

12.5 Reporting Procedures to NIH IRB and FMPOS EC

12.5.1 Expedited Reporting to the NIH IRB and FMPOS EC

Non-compliance: Any actual or suspected non-compliance by any investigator or entity associated with the protocol must be reported by the NIH PI/designee within 7 calendar days of any investigator or individual associated with the protocol first becoming aware, unless otherwise indicated in this policy.

Non-NIH IRB determinations of serious and/or continuing noncompliance about an NIH investigator: If the NIH is relying on a non-NIH IRB and the Reviewing IRB makes a determination of serious and/or continuing non-compliance regarding an NIH investigator, then, even if the determination has already been provided to OHSRP either directly or via the NIH Institutional Official (IO)/designee, the NIH PI/designee must report this in iRIS within 7 calendar days of any member of the research team being notified of the determination by the Reviewing IRB. The NIH PI must provide the OHSRP office of Compliance and Training with documentation from the Reviewing IRB unless this documentation has already been provided directly to the office by the Reviewing IRB or via the IO.

Major Deviation: A deviation must be reported within 7 calendar days of an investigator becoming aware of an actual or suspected deviation. Although protocol deviations are also non-compliance, these should only be reported once as deviations.

Unanticipated Problem (UP): A UP must be reported within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

Death: Any death of a research subject that is possibly, probably or definitely related to the research must be reported within 24 hours of an investigator becoming aware of the death.

New information that might affect the willingness of a subject to enroll or remain in the study should be reported within 7 calendar days of an investigator first becoming aware.

Any suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency must be reported within 7 calendar days of an investigator becoming aware.

Investigators must provide the following information to the IRB in summary format at the time of continuing review: Minor protocol deviations; Adverse events and Serious Adverse Events that do not meet the definition of an UP.

Investigators are required to immediately (i.e., no longer than 10 days) report SAEs or UADEs to the study sponsor and, if also an actual or suspected UP, to the IRB within 7 calendar days of an investigator becoming aware.

12.5.2 Annual Reporting to the NIH IRB and FMPOS EC

The following items will be reported to the NIH IRB and FMPOS EC in summary format at the time of Continuing Review: Minor protocol deviations; Adverse events and Serious Adverse Events that do not meet the definition of an UP.

12.6 Additional Investigator Reporting Responsibilities to the Local IRB (NIH 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.

12.7 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. Quarterly summaries regarding pregnancy (maternal and neonatal outcomes) will be submitted to the board for review on an ongoing basis. 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).

12.8 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.

12.9 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.

12.10 Pausing Criteria for the Main Vaccine Phase

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.

12.11 Pausing Criteria for the Pregnancy and Offspring Follow-up Phase

The PI will closely monitor blinded study data as they become available and will make determinations regarding the presence and grading of Aes, in particular defined safety and protective efficacy outcomes in pregnant women and their offspring, as defined by the protocol. These Aes and safety and protective efficacy outcomes will be evaluated by the study team, Sponsor, and DSMB on a regular basis defined at the start of the study, for occurrence of events outside of the expected background rate in the community.

If a concern arise during the course of vaccination, all vaccinations will be halted until DSMB review has been held. Post vaccination, an aggregate report of these events will be provided on a quarterly basis for review by the Sponsor, IRB/EC, and DSMB. If there's an identified concern about pregnancy outcomes, all enrolled women will be provided an update of the concern and any additional recommendations associated with the concerns as the information becomes available.

12.11.1 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 of awareness of, 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 NIH 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.

12.11.2 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.

12.12 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.

12.12.1 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 (NIH IRB, FMPOS EC). The IND sponsor will report this to the DSMB.

12.12.2 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.

12.13 Discontinuation of Study

Sanaria, Inc. as the study sponsor, the NIH 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

12.14 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 pausing of the subject from further vaccinations until the subject is reviewed by the study Sponsor, DSMB .. 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 not receive 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.

12.14.1 **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. Subjects withdrawn before the first vaccination may be replaced.

12.15 **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.

In general, unless requested by the PI or designee, sponsor, sponsor medical monitor, independent medical monitor, or DSMB, pregnancies will not result in unblinding of subjects involved in the study.

Subjects who are unblinded will be encouraged to remain in the study to be followed for safety regardless of their indication for unblinding.

Scheduled unblinding, for all study participants – pregnant or not pregnant, will occur at the completion of the subject's final malaria follow-up visit in Year 2 (study visit 561; final study visit of Year 2 exploratory efficacy endpoint) as outlined in **Appendix A.** Individuals directly continuing AE assessment or performing assays for exploratory endpoints will remain blinded to individual randomization until assessment or assays are completed.

The study Sponsor, Sanaria, Inc., requested in writing an unscheduled unblinding, to support future PfSPZ Vaccine studies, to occur prior to planned unblinding of the protocol on study day 561 (anticipated Feb 2021). This unblinding was requested to only include Year 1 data from screening to study day 197 (from July 2019 to Feb 2020) (final study visit of Year 1 secondary efficacy endpoint/malaria follow-up) and include the following information: enrollment data, demographics, drop outs (through study day 197), solicited (local and systemic) AEs through study day 36, unsolicited AEs through study day 57, laboratory AEs through study day 57, and SAEs through study day 197. Summary of pregnancies and miscarriages through study day 197 were provided by the study team. This request was reviewed by the scientific review committee, DSMB, NIH IRB, and FMPOS EC prior to implementation. LMIV data manager provided a blinded report for collation of this data. MRTC and LMIV staff were not involved in the transmission, review, or discussion of any unblinded results. Unblinding data was transmitted directly from the unblinded study statistician (or designee not involved with direct study participant care or evaluation) to the Sponsor for submission to the FDA.

Given that no additional study product will be administered in the continued follow-up of previous participants, no scheduled DSMB meeting will be set up unless necessary as outlined in **Section 12.16.3** or at the request of the DSMB, but during the final DSMB meeting for the overall study, results from this extension will also be presented.

12.16 Safety Oversight

12.16.1 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.

12.16.2 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.

12.16.3 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 **Section 12.7**. 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).

13 CLINICAL MONITORING

13.1 Site Monitoring Plan

As per International Conference on Harmonization (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.

14 STATISTICAL CONSIDERATIONS

14.1 Endpoints and Statistical Methods

14.1.1 Safety Analysis

Safety will be assessed by line listing and tables at the individual level. Safety will be additionally assessed over women who become pregnant during the study and their newborns. These safety profiles will be reviewed in light of baseline rates in the targeted population for pregnancy outcomes defined in an ongoing pregnancy registry at the study site. Other concerning trends will be investigated as needed or requested by the PI, ISM, IND sponsor, and/or DSMB. Group comparisons, between each vaccine arm and the control arm, may be performed in terms of the proportion of AE and SAE adopting the as-treated analysis and the per protocol analysis.

14.1.2 Vaccine Efficacy Analysis

Primary efficacy analysis will be based on time to infection during the first year post vaccination. The time to infection, which is defined as the time to the first positive blood smear. Each vaccine arm will be compared with the control arm by logrank test for interval-censored data (using the interval package in R). Vaccine efficacy (VE) will be estimated assuming a Weibull model and allowing for interval censoring (survival package in R).

The proportions of infection will be also compared between each vaccine arm with the control arm by the exact binomial test, or the Exact Cochran-Mantel-Haenszel test (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 a modified ITT and a per-protocol analysis. Under the modified ITT analysis, subjects receiving at least one vaccination will be included and will be analyzed according to the assigned arm. Under the per-protocol analysis, subjects that drop out prior to receiving the full number of vaccinations will not be included in the analysis and they will be analyzed according to the regimen actually received.

Exploratory analysis on efficacy will also be conducted on the subset of women who become pregnant during followup with respect to *P.falciparum* blood stage infection and *P.falciparum* placental infection. The timing of pregnancy and number of prior pregnancies may be included as a confounder in the efficacy analysis. Exploratory efficacy analysis may also be performed to compare the two vaccine dose arms.

Additional exploratory analyses may be completed looking at other malaria infection endpoints, such as symptomatic malaria episodes, malaria infection detected by PCR, gametocytemia, and non-*P. falciparum* infections.

Similar efficacy analyses as above will be performed over the Year 2 data at the end of Year 2 follow-up. In addition, we will assess the protective efficacy over Year 1 and Year 2 combined.

14.1.3 **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.

14.1.4 **Pregnancy and Neonatal Outcomes**

As noted, prior special attention will be given to outcomes for women who become pregnant during the study and their newborns, and will be reviewed in light of background rates for pregnancy outcomes defined in an ongoing pregnancy registry at the study site. Given the pregnancy rates seen thus far during the Pregnancy Registry (#17-I-N018), we expect the following occurrences of pregnancies and deliveries over the course of the study as outlined in **Table 12** and **Table 13**.

Table 12: Number of Expected Pregnancies Post PfSPZ Vaccine or Normal Saline Vaccination

	Aug 2019	Sep 2019	Oct 2019	Nov 2019	Dec 2019	Jan 2020	Feb 2020	Mar 2020	Apr 2020	May 2020	Jun 2020	Jul 2020	Aug 2020	Total
Estimated PfSPZ Vaccine (n=200)	1 (0.5%)	2 (1 %)	1 (0.5%)	2 (1 %)	3 (1.6%)	4 (2.1%)	6 (3.3%)	8 (4.6%)	10 (6.1%)	9 (5.8%)	9 (6.2%)	8 (5.8%)	8 (6.2%)	71 (35.5%)
Estimated Normal Saline (n=100)	0 (0.0%)	1 (1%)	0 (0.0%)	1 (1%)	1 (1%)	2 (2.1%)	3 (3.3%)	4 (4.5%)	5 (6%)	5 (6.4%)	4 (5.5%)	4 (5.8%)	4 (6.2%)	35 (35%)
Actual Totals from Current Protocol[A]	0/288 (0.0%)	0/288 (0.0%)	6/288 (2.1%)	5/282 (1.8%)	10/277 (3.6%)	14/267 (5.2%)	15/254 (5.9%)	19/245 (7.8%)	19/232 (8.2%)	8/214 (3.7%)	6/209 (2.9%)	14/203 (6.9%)	13/191 (6.8%)	129/288[B] (44.8%)
Actual Number of Pregnancy Loss from Current Protocol	0	0	0	0	0	1	6	6	1	3	0	2	1	19[C]

+Depo-Provera = median delay before conception was 5.5 months + 4 months after discontinuing; IUD = median delay before conception was 4.5 months after discontinuing.

[A]Eligible Women = 288 women received Vaccination #3. Not included are: 4 enrolled, but not vaccinated, women who became pregnant and 1 woman who became pregnant post Vaccination #2.

[B]129 pregnancies occurred in 120 individual women during this time period. 9 women became pregnant twice during this time period

[C]20 miscarriages occurred in 19 individual women during this time period

Table 13: Number of Expected Children Post PfSPZ Vaccine or Normal Saline Vaccination

	May 2020	Jun 2020	Jul 2020	Aug 2020	Sep 2020	Oct 2020	Nov 2020	Dec 2020	Jan 2021	Feb 2021	Mar 2021	Apr 2021	May 2021	Total
Estimated PfSPZ Vaccine (n=200)	1	2	1	1	3	3	5	7	7	8	7	7	6	58
Estimated Normal Saline (n=100)	0	1	0	2	1	1	3	3	4	4	3	4	3	29
Actual Deliveries from Current Protocol	0	4	7	13	10	9*	14	17	10	6	10	15	15	130

+Assuming ~15-20% baseline pregnancy loss; note this estimated loss may be underrepresented but will be adjusted as more data is collected under #17-I-N018.

[A]Only women who became pregnant post Vaccination #3 included in this table. Not included are: 4 enrolled, but not vaccinated, women who became pregnant and 1 woman who became pregnant post Vaccination #2.

*Excludes two stillbirths.

14.2 Sample Size Consideration

Three hundred subjects will be enrolled and randomized 1:1:1 to the control and the two vaccination arms. All subjects will contribute to safety evaluation at least up to certain time points. For safety evaluation, with 100 subjects in each arm, there is >90% probability of observing at least one adverse event if the adverse event rate is no less than 0.023. Previous studies on the study vaccine have indicated low adverse event rate associated with the vaccine. The goal of safety analysis is to establish the safety profile of vaccine arm.

Sample size consideration primarily targets vaccine efficacy evaluation basing on the comparison of each vaccine arm with the control arm. The null hypothesis is VE=0 and the alternative hypothesis is VE is not equal to zero. If the annual drop-out rate is between 15% and 25%, there will be 75 to 85 subjects available in each arm for efficacy evaluation one year after study initiation. With 80 samples in each arm, the study is capable of detecting the protective efficacy of vaccine with over 80% power if VE is 45% or higher and the background infection rate is ≥0.5 (see **Table 10**). These calculations are based on Cox proportional-hazard model at two-sided significance level of 0.05, which follows a normal approximation to the logrank test

without adjustment for interval censoring [Reference: Fundamentals of Biostatistics, Bernard Rosner, 2006].

Table 14: Power for group-wise comparison based on logrank test on time to infection

Sample size per arm	Background infection rate	Vaccine Efficacy	Power
75	0.5	0.45	81
75	0.5	0.3	46
75	0.5	0.25	34
75	0.6	0.45	92
75	0.6	0.3	60
75	0.6	0.25	45
75	0.7	0.45	98
75	0.7	0.3	75
75	0.7	0.25	60
80	**0.5**	**0.45**	**84**
80	**0.5**	**0.3**	**48**
80	**0.5**	**0.25**	**36**
80	**0.6**	**0.45**	**94**
80	**0.6**	**0.3**	**63**
80	**0.6**	**0.25**	**48**
80	**0.7**	**0.45**	**98**
80	**0.7**	**0.3**	**78**
80	**0.7**	**0.25**	**62**
85	0.5	0.45	86
85	0.5	0.3	51
85	0.5	0.25	37
85	0.6	0.45	95
85	0.6	0.3	65
85	0.6	0.25	50
85	0.7	0.45	99
85	0.7	0.3	80
85	0.7	0.25	65

14.3 **Randomization and Blinding**

Eligible participants will be randomized to study arms using block randomization of sequentially-numbered study ID numbers that are generated sequentially for those participants who meet inclusion criteria after screening. Randomization codes will be generated by study statisticians and provided to the pharmacist by direct delivery in sealed, opaque envelopes or

electronically using a secure email server. The study is blinded to participants and study staff involved with assessing all clinical and laboratory outcomes.

14.4 Interim Analysis

No interim analysis will be performed in this trial. The primary analysis on efficacy will be performed after the first peak transmission season, approximately in January 2020. The same efficacy analysis methods will be applied to the interim analysis in both modified ITT and the per protocol cohorts. This analysis will be performed to assist regimen selection in future studies. The analysis will completed by the study statistician and will be reviewed by LMIV senior investigators 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 and completion of study related assays. In this way, they analysis results will not affect the secondary analysis on efficacy that will be performed after the end of the second transmission season.

In October 2020, the study Sponsor (Sanaria, Inc) requested an unblinded analysis on safety, immunogenicity, efficacy, and pregnancy rates/outcomes be made available from Year 1 of the study (through primary efficacy endpoint, study day 197) to share with the FDA which was approved by Scientific Review, NIH IRB, FMPOS EC, and DSMB and was submitted to the FDA by the Sponsor.

15 Human Subject Protections and Ethical Obligations

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

15.1 Institutional Review Board

A copy of the protocol, informed consent forms (ICFs), and other study-related information 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.

15.2 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.

15.2.1 **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.

15.2.2 **Individual Informed Consent in Mali**

WOCBP 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 orally in cases of illiteracy. WOCBP 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 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 informed consent that need further review with subject. This will help ensure that the subject has sufficient understanding before the consent form is signed. The subject may either sign the consent form immediately or later after further consideration. 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.

For rescreening, no Malaria Comprehension Exam will be required, but individual informed consent will be obtained from each participant choosing to continue as described above.

15.3 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.

16 POTENTIAL RISKS AND BENEFITS

For any clinical trial, the risk for potential subjects should be weighed against the benefit. PfSPZ Vaccine has been shown in previous studies to provide protection from malaria infection in vaccinated individuals at similar dosing regimens and schedules as proposed here. It is unknown if there is continued protective efficacy or clinical benefit in a woman who becomes pregnant following vaccination and within their offspring.

Risks to the subjects are associated with venipuncture, immunization, or drug administration. These risks are outlined below:

16.1 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 is 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.

For follow-up into 2022-2023 malaria transmission season, venipuncture is the only risk to women re-enrolled.

16.2 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.

16.3 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 the course of conducting PfSPZ Vaccine trials 7 participants have become pregnant during the course of the study. Two pregnancies were carried to term; although one of the 2 pregnancies was complicated by intrauterine growth retardation (IUGR) and some subsequent fetal abnormalities, these complications were not thought to be related to vaccine. One pregnancy resulted in early fetal demise – in this case the date of vaccine administration was approximately the same as the estimated date of conception and the event was considered possibly related to

vaccine. Two pregnancies were electively terminated by the study participant. Two additional pregnancies are ongoing and appear to be progressing normally.

Details of each pregnancy are provided below.

1. **Bethesda, MD (USA):**
   G1P0 woman who had a positive screening serum pregnancy test on 1 July 2014 (43 days after last immunization), confirmed by a repeat test on 2 July 2014. Her LMP was 12 June and reportedly normal. Her 4th of 4 immunizations with 2.7x10^5 PfSPZ of PfSPZ Vaccine was 19 May 2014. Her previous pregnancy test on 2 June was negative and she underwent controlled human malaria infection (CHMI) on 3 June. She remained negative for *P. falciparum* parasitemia by blood smear and PCR throughout the subsequent 28-day monitoring period. As this was an unplanned pregnancy, the participant elected to terminate the pregnancy on 7 July 2014.
2. **Equatorial Guinea:**
   19 year old G3P2 woman was found to have a positive urine pregnancy test on 4 January 2017 after reporting her LMP was 13 November. Her previous urine pregnancy test was negative on 1 December 2016. She had initiated oral contraceptives after the 1 December visit and also received her first dose of 2.7x10^6 PfSPZ of PfSPZ Vaccine. After her positive pregnancy test no further vaccine was administered. On 17 January 2017 (estimated gestational age 9 2/7 weeks) she underwent transvaginal ultrasound which showed a 6-week embryo without cardiac activity; these findings were confirmed on a repeat ultrasound a week later. The products of conception were evacuated using misoprostol without further complication.

   18 yo G1P0 woman who presented to her local clinic on 30 August 2017 with nausea, vomiting, generalized weakness and a last menstrual period on 18 July 2017 (90 days after her last dose of vaccine). Her urine pregnancy test was positive. She reported no use of contraception. Her third and final dose of 1.8x10^6 PfSPZ of PfSPZ Vaccine was administered 19 April 2017. The participant's vomiting progressed and she was admitted for IV fluids and symptom control with a diagnosis of hyperemesis gravidarum of moderate severity. Her symptoms were successfully managed and there were no further complications during the pregnancy. She delivered a healthy baby girl at 37 4/7 weeks weighing 2880 grams. Examination of the infant at 1 month was normal except for an umbilical granuloma.

   15 yo G1P0 woman was found at a prevaccination assessment visit 18 April 2017 to have a positive urine pregnancy test. She had previously received 1.8x10^6 PfSPZ of PfSPZ Vaccine on 8 December 2016 and 25 February 2017. By ultrasound her estimated date of conception was 7 April 2017 (41 days after vaccination) with estimated delivery date of 29 December 2017. Fetal ultrasounds were normal at 16 and 24 weeks. At 30 5/7 weeks there was evidence of IUGR followed by onset of gestational hypertension and proteinuria at 33 weeks. The

infant was delivered by cesarian section at 33 2/7 weeks with a weight of 1300 grams, Apgar scores of 8 and 9. The volunteer had no further complications and recovered fully. The infant was initially hypoglycemic but responded to treatment; no evidence of neonatal sepsis was found. The baby was discharged after 3 days and gained weight appropriately at home. A patent ductus arteriosus (PDA) was identified on echocardiogram on 4 December and treated with 2 days of ibuprofen; repeat echocardiograms dated 31 January and 30 April 2018 showed the PDA had decreased in size. Also noted were moderate tricuspid insufficiency and a patent foramen ovale. Cardiac chamber sizes and systolic function were normal. The child also had a protuberant right inguinal hernia and underwent bilateral inguinal herniorrhaphies under general anesthesia on 26 December 2017 without complication.

3. **Tanzania:**
   26 year old G1P0 woman who had a positive urine pregnancy result on a home test kit approximately 20 days after starting a 4-dose series of immunizations with $9x10^5$ PfSPZ every other day. Urine pregnancy testing prior to immunizations had been negative. She subsequently self-administered misoprostol vaginal suppositories. When she presented for her booster immunization pre-assessment on day 26 her urine pregnancy test was positive and she was found by ultrasound to have had an incomplete abortion. She subsequently underwent a dilatation and evacuation without further complications.

4. **Mali:**
   25 year old G4P3 woman who presented to the clinic on 8 January 2019 with nausea, dizziness, headache, myalgias and a missed menstrual period. A serum pregnancy test was positive. Her third and final dose of investigational product was administered 18 September 2018 (112 days previously); her last injection of medroxyprogesterone was 19 July 2018. No further problems have been reported for this participant. The estimated due date is 4 August 2019.

   21 year old G4P3 woman who presented to the clinic on 19 January 2019 with mild abdominal pain, headache, dizziness, maylgias and feverishness x 3 days plus 2 months of amenorrhea. Malaria RDT was negative; a serum pregnancy test was positive. Her third and final dose of investigational product was 12 September 2018 (129 days previously); her last injection of medroxyprogesterone was 13 August 2018. No further problems have been reported for this participant. The estimated due date is 26 August 2019.Of note from the obstetric history – all 3 prior births were live births but the first child died after 1 day of unknown causes and the 2^{nd} child died after 5 days of unknown causes.

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, women of child bearing potential must use parental injectable contraceptive or have a pre-existing implantable or intrauterine device at least 21 days prior to receipt of Sanaria® PfSPZ Vaccine injection.

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 one.

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.

16.4 **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 of administration technique and have been local injection reactions as noted above in **Section 15.2**.

16.5 **Antimalarial Medication**

Subjects will be treated with a registered, oral, proven, and highly efficacious treatment during the course of this study per standard clinical practice. These medications will be tracked closely, similar to study specific products, but are not considered investigational for this study as they are standard of care treatments for uncomplicated and complicated malaria.

16.5.1 **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, though it is currently recommended by WHO for treatment of uncomplicated malaria during the second and third trimester. 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, AL 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 (e.g. barrier methods, abstinence).

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

16.6 **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.

16.7 **Pregnancy**

Malaria infection can have adverse effects on both the pregnant mother and fetus. Women who are pregnant at the time of enrollment are excluded from the study.

Pregnancy testing is performed during screening and throughout the vaccination period, including prior to each PfSPZ Vaccine or placebo vaccine via DVI and prior to AL dosing. Pregnancy prevention counseling and compliance is reinforced at every clinic visit (see **Appendix A**) throughout the vaccination period and for 28 days post vaccination #3.

If clinical history indicates recent sexual activity that may lead to pregnancy, or history or symptoms suggestive of pregnancy, a serum β-HCG test will be performed and results reviewed by a study investigator prior to the subject continuing in the study.

If a subject becomes pregnant during the first year of the study (2019; through study day 365), the subject will not be withdrawn from the study but no further investigational products will be administered. The Sponsor and NIH IRB/FMPOS EC will be notified of the pregnancy as outlined in **Section 12.7**. 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, as outline in **Appendix A,** which will include obtaining blood for safety labs (if indicated), blood smear +/- PCR samples, and periodic research samples. This study schedule may be altered at the investigator's discretion if any concern that the blood draws would impact the health of the mother or the fetus. The mother will be invited to continue follow up until the end of pregnancy, and to enroll her newborn and herself in one year of additional follow up, in order to document the pregnancy and neonatal outcomes. Pregnant women will be seen at antenatal consultation including at least one visit during the first, second, and third trimester. In addition to their usual prenatal care, pregnant women will be invited to consult with study clinicians on a monthly basis for a clinical assessment including blood pressure measurement.

The newborn will followed-up actively and passively. Active surveillance will be completed with in-person visits every two months for 12 months as well as additional telephone consultations completed every two weeks through 6-months of life. Passive follow-up will be done through the availability of the study clinicians 24 h/day and 7 days as week to assess the pregnant women and newborn through the follow-up period. For new pregnancies detected in Year 2 of follow-up (study day 393 onwards), newborns will only have delivery outcomes collected (study visit C0) and will not be followed beyond this visit.

If a woman miscarries, she will be permitted to continue on the study as a non-pregnant subject and will join that study schedule on the closest estimated upcoming visit. If she becomes pregnant again while on study, she will then follow the study schedule depending on the timing of the detection of her pregnancy (Year 1, prior to study day 393, versus Year 2, study day 393 onwards).

Post-partum, women whose pregnancy was determined in Year 1 will be followed for ~12 months post delivery. For women who become pregnant in Year 2, she will only be followed for ~1-2 month's post delivery.

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

16.8 Risk to the Community

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

17 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 15**, 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 equals US $ 6 or US $ 3 for each visit depending on the time spent by the volunteer. Volunteers will receive US $ 6 when they have to do more evaluation process so spend more time at the center, and US $ 3 when the process and time are less. Volunteer compensation payments will be made periodically throughout of the test.

Table 15: Estimated Compensation for the Study

Study Group(s)	US Dollar Equivalent	Rice or Millet Dispensed (Local Currency [CFA]
WOCBP Vaccine Phase	$186	93000 F CFA
Pregnant Women[1]	$69	34500 F CFA
Offspring	$27	13500 F CFA
Non-pregnant Year 2	$78	39000 F CFA
Non-pregnant + Pregnant Women Year 4	$78	39000 F CFA

*Assuming currency exchange rate of USD $1= 500 CFA; this may be adjusted due to economic conditions.

[1]Compensation during pregnancy may be more if pregnant women agree to non-pregnant visits (every 2 weeks) during the malaria season.

18 DATA HANDLING AND RECORD KEEPING

18.1 Data Capture and Management

In Mali, the study data will be collected directly into a study specific DataFax electronic database. Data from CRFs will be collected directly from subjects during study visits and telephone calls. Any type of corrections to electronic will be initialed and dated by the person making the correction. All final electronic CRFs will reviewed by the Investigator or designee.

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, what was changed, and automated downloads of the electronic database will be completed daily. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

18.2 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.

18.3 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.

18.4 Protocol and ICF 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 and/or ICF will be submitted to the sponsor for review and then to the participating IRBs and FDA as a protocol and/or ICF 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.

19 Role of NIH Investigators

MRTC and NIH/NIAID/LMIV investigators work collaboratively on this clinical trial, however, NIH investigators are not directly engaged in clinical research, such as recruiting, consenting, assessing adverse events, and/or administering drug or vaccine. NIH investigators do have access to personal identifying information from this trial.

20 Role of Collaborators

Dr. Blair Wylie will be providing her experience and expertise to assist with study design, protocol training and implementation, standardization of obstetric related evaluations, and discussion with the study team regarding adverse events (on a de-identified basis).

21 APPENDICES

Appendix A: Study Schedule

Appendix A: Clinic and Laboratory Procedures for Main Vaccination Phase																	
		Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Unscheduled -- Blood Smear Positive (B)
		Study Day	-56	-14	1	2	4	8	9	11	15	22	29	30	32	36	N/A
		Days post-PfSPZ Vaccine	-56	-14	0	1	3	0	1	3	7	14	0	1	3	7	N/A
		Weeks post-PfSPZ Vac #1	-8	-2	0			1			2	3	4			5	N/A
		Visit windows (days)		±7		0	±1	±2	0	±1	±7	±3	±3	0	±1	±2	
			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	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																	
Complete medical history/ physical			x														
Informed consent			x														
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)
Interim clinical evaluation				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
Conmed review			x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
Unblinding																	
AL dose				X							X	x					
PfSPZ Vaccine or Placebo					x			x					x				
LABORATORY PROCEDURES	Designated Laboratory	Tube Type															
Screening/Safety Labs and procedures																	
CBC with differential + sickle cell screening (day -14 only)	MRTC CAP lab/UB	EDTA	2	2	2		2	2		2	2		2		2	2	
ALT, Cr + HIV		SST	3	3	3		3	3		3	3		3		3	3	
Urine dipstick or UA		Urine Container	x														
Urine/Serum Pregnancy Test (A)		Urine Container or SST	x	x	x			x			X		x				(X)
ECG	NIH/Mali Cardiology	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
Peripheral Blood Smear		EDTA		0.5	0.5			0.5			0.5		0.5			0.5	
Research Assays																	
Humoral Assays	MRTC/ LMIV	SST		10							5						
Cellular Assays		NaHep		10							10					10	
Ex Vivo Assays		EDTA		0.5			0.5				0.5					0.5	0.5
Parasite Purification (CF11)		EDTA															4
Transcriptional Assays		Nucleic Acid Stabilizer		1							1				1		
Daily total			5	27.5	6	0	5.5	6	0	5	22.5	0	6	0	6	16.5	5
Study cumulative total			5.0	32.5	38.5	38.5	44.0	50.0	50.0	55.0	77.5	77.5	83.5	83.5	89.5	106.0	

(A) If serum pregnancy testing required, an additional 3mL of blood may need to be drawn

(B) For unscheduled blood smear positive visits -- for first positive blood smear, subjects should return within 48 hours to blood draw; no need to repeat blood smear given positive BS prompting visit unless clinically indicated; first positive BS = first positive post vaccination (preference given post vaccination #3) or new positive BS occurring >14 days after recent anti-malarial treatment or new positive BS occurring >28 days after negative BS regardless of treatment; visit is optional, with priority for completing visit for first positive BS post vaccination #3

		Clinic visits	15	16	17	18	19	20	21	22	23	24	25	26	26	27	28	29	30	31	Unscheduled – Blood Smear Positive (B)
		Study Day	43	57	71	85	99	113	127	141	155	169	183	197	225	253	281	309	337	365	N/A
		Days post-PfSPZ Vaccine	14	28	42	56	70	84	98	112	126	140	154	168	196	224	252	280	308	336	N/A
		Weeks post-PfSPZ Vac #1	6	8	10	12	14	16	18	20	22	24	26	28	32	36	40	44	48	52	N/A
		Visit windows (days)	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	
			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	Post Vax F/U	Post Vax F/U	Post Vax F/U	Post Vax F/U	Post Vax F/U	Post Vax F/U	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																					
Complete medical history/ physical																					
Informed consent																					
Malaria Comprehension Exam																					
Pre-test/Post-test HIV counseling																					
Pregnancy Prevention Counseling			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
Counsel review			X	X	X	X	X	X	X	X	X	X	x	x	X	X	X	X	X	X	X
Unblinding																					
AL dose																					
PfSPZ Vaccine or Placebo																					
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																			
Screening/Safety Labs and procedures																					
CBC with differential + sickle cell screening (day -14 only)	MRTC CAP lab/UB	EDTA																			
ALT, Cr + HIV		SST																			
Urine dipstick or UA		Urine Container																			
Urine/Serum Pregnancy Test (A)		Urine Container or SST			X		X		X		X		x		X	X	X	X	X	X	(X)
ECG	NIH/Mali Cardiology	N/A																			
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
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	5	2.5				2.5				2.5									
Cellular Assays		NaHep	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									
Daily total			16.5	15	1	1	1	13.5	1	1	1	15	1	1	1	1	1	1	1	1	5
Study cumulative total			122.5	137.5	138.5	139.5	140.5	154	155	156	157	172	173.5	174.5	175.5	176.5	177.5	178.5	179.5	180.5	
(A) If serum pregnancy testing required, an additional 3mL of blood may need to be drawn																					
(B) For unscheduled blood smear positive visits – for first positive blood smear, subjects should return within 48 hours to blood draw; no need to repeat blood smear given positive BS prompting visit unless clinically indicated; first positive BS = first positive post vaccination (preference given post vaccination #3) or new positive BS occurring >14 days after recent anti-malarial treatment or new positive BS occurring >28 days after negative BS regardless of treatment; visit is optional, with priority for completing visit for first positive BS post vaccination #3																					

Appendix A: Clinic and Laboratory Procedures for Pregnant Women

		Trimester (A)	TBD	1st (B)			2nd			3rd			N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Month post Delivery	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0 (Delivery)	2	4	6	8	10	12
		Study Day	P0	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
		Visit windows (days)	0	±14	±14	±14	±14	±14	±14	±14	±14	±14	±2	±21	±14	±14	±14	±14	±14
			Enroll	Preg F/U	Preg F/U	Preg F/U	Preg F/U	Preg F/U	Preg F/U	Preg F/U	Preg F/U	Preg F/U	Delivery	Post Delivery F/U	Post Delivery F/U	Post Delivery F/U	Post Delivery F/U	Post Delivery F/U	Post Delivery F/U
CLINICAL PROCEDURES																			
Complete medical history/ physical			X																
Informed consent for pregnancy follow-up			X																
Interim clinical evaluation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy outcome assessment			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Informed consent for offspring follow-up			(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)					
Unblinding ©												(X)							
LABORATORY/IMAGING PROCEDURES	**Designated Laboratory**	**Tube Type**																	
Labs and procedures																			
CBC with differential	MRTC CAP lab/UB	EDTA		2			2			2			2						
ALT, Cr		SST		3			3			3									
Glucose		SST																	
Blood type		EDTA		X															
Serologies (syphilis, rubella, toxoplasmosis)		SST		3															
Urine dipstick or UA		Urine Container		X			X			X									
Urine/Serum Pregnancy Test		Urine Container or Lithium Hep	X	X	X	X	X												
Prenatal Ultrasound	Mali	N/A	(X)		X			X			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
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
Research Assays (D)																			
Humoral Assays	MRTC/ LMIV	SST		5						5			5			5			5
Cellular Assays		NaHep																	
Ex Vivo Assays		EDTA		0.5						0.5			0.5			0.5			0.5
Parasite Purification (CF11)		EDTA																	
Transcriptional Assays		PaxGENE		1						1			1			1			1
Placenta		Other											X						
Cord Blood		SST, EDTA, PaxGene											X						
Total			0	15.5	1	1	6	1	1	12.5	1	1	9.5	1	1	7.5	1	1	7.5
Study cumulative total			0	15.5	16.5	17.5	23.5	24.5	25.5	38	39	40	49.5	50.5	51.5	59	60	61	68.5

(A) Depending on the timing of pregnancy diagnosis, at least 1 but up to 3 scheduled visits may be completed per trimester. Procedures and labs as noted will be completed at each visit during that trimester unless otherwise noted; if visits occur during the 24 week malaria infection follow-up, women will be asked to provide safety and malaria labs per Vaccine schedule

(B) Standard pregnancy labs will be coordinated with the subject's identified OB/GYN; if not drawn at OB/GYN labs will be drawn in the clinic and shared with the OB/GYN + patient. If drawn at OB/GYN, we will not repeat the labs but ask for copy of results from subject's OB/GYN

(C) Unblinding will occur on estimated study day 561 for all study participants regardless where they are in the time course of their pregnancy

(D) If a woman delivers early and research labs during third trimester have been collected within 8 weeks' prior to delivery, at the investigator discretion, those research labs do not need to be repeated at time delivery (excluding placenta and cord blood collection); CBC with differential, qPCR and peripheral BS will be collected.

Appendix A: Clinic and Laboratory Procedures for Neonates/Infants

													Unscheduled -- Blood Smear Positive
		Months post Birth	N/A	0.5, 1, 1.5	2	2.5, 3, 3.5	4	4.5, 5, 5.5	6	8	10	12	
		Study Day	C0	CT1, CT2, CT3	C1	CT4, CT5, CT6	C2	CT7, CT8, CT9	C3	C4	C5	C6	N/A
		Visit windows (days)	0	±7	±21	±7	±14	±7	±14	±14	±14	±14	
			Enroll (B)	Phone F/U	F/U	Phone F/U	F/U	Phone F/U	F/U	F/U	F/U	F/U	Unscheduled Visit Positive BS
CLINICAL PROCEDURES													
Complete medical history/ physical			X										
Interim clinical evaluation (including weight, length, head circumference)					X		X		X	X	X	X	X
Neonatal/Infant outcome assessment			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X
EPI vaccine review			X		X		X		X	X	X	X	
Developmental screening									X			X	
LABORATORY/IMAGING PROCEDURES	**Designated Laboratory**	**Tube Type**											
Labs and procedures													
Hemoglobin	MRTC CAP lab/UB	EDTA							0.5			0.5	
Hemoglobin typing		EDTA										0.5 (A)	
Malaria Infection Assays													
qPCR	MRTC CAP lab/LMIV	EDTA			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	
Research Assays													
Humoral Assays	MRTC/ LMIV	SST							3			3	
Cellular Assays		NaHep											
Ex Vivo Assays		EDTA							0.5			0.5	0.5
Parasite Purification (CF11)		EDTA											4
Transcriptional Assays		PaxGENE							1			1	
Total			0	0	1	0	1	0	6	1	1	6.5	5
Study cumulative total			0	0	1	0	2	0	8	9	10	16.5	

(A) Hemoglobin typing can be collected at any time point; preference is for visit with venous blood draws (C3, C6 with C6 preferred given older age/weight). But if a subject may be moving or leaving the study area, this can be collected prior to departure at any visit

(B) Infants enrolled from pregnancies detected in Year 2 (study day 393 onwards) will only complete study visit C0

Year 2 Follow-up: Non-pregnant Follow-Up

Appendix A: Clinic and Laboratory Proceduresfor Year 2																
		Clinic visits	32	33	34	35	36	37	38	39	40	41	42	43	44	Unscheduled -- Blood Smear Positive (B)
		Study Day	393	407	421	435	449	463	477	491	505	519	533	547	561	N/A
		Days post-PfSPZ Vaccine	364	378	392	406	420	434	448	462	476	490	504	518	532	N/A
		Weeks post-PfSPZ Vac #1	56	58	60	62	64	66	68	70	72	74	76	78	80	N/A
		Visit windows (days)	±56	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	
			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	Malaria F/U and Unblinding Visit	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																
Informed consent/ Addendum Consent			X													
Focused clinical evaluation (if clinically indicated) (A)			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Unblinding															X	
LABORATORY PROCEDURES	Designated Laboratory	Tube Type														
Urine/Serum Pregnancy Test (B)		Urine Container or SST	X	X	X	X	X	X	X	X	X	X	X	X	X	(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
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	
Research Assays																
Humoral Assays	MRTC/ LMIV	SST	2.5	5	2.5				2.5				2.5		2.5	
Cellular Assays		NaHep	10.0	10	10				10				10		10	
Ex Vivo Assays		EDTA	0.5	0.5	0.5								0.5		0.5	0.5
Parasite Purification (CF11)		EDTA														4
Transcriptional Assays		PaxGENE (or similar tube type)													1	
Daily total			14	16.5	14	1	1	1	13.5	1	1	1	14	1	15	5
Study cumulative total			180.5	197	211	212	213	214	227.5	228.5	229.5	230.5	244.5	245.5	260.5	

(A) Given >1 year since study product, clinical exam including vital signs will only be completed if clinically indicated.

(B) If serum pregnancy testing required, an additional 3mL of blood may need to be drawn

(C) For unscheduled blood smear positive visits -- for first positive blood smear, subjects should return within 48 hours to blood draw; no need to repeat blood smear given positive BS prompting visit unless clinically indicated; first positive BS = first positive post start of Year 2 or new positive BS occurring >14 days after recent anti-malarial treatment or new positive BS occurring >28 days after negative BS regardless of treatment; visit is optional, with priority for completing visit for first positive BS post start of Year #2

Year 4 Follow-up/3-Years Post Initial Vaccination: Non-pregnant + Pregnant Follow-Up

Appendix A: Clinic and Laboratory Proceduresfor Year 4																
		Clinic visits	45	46	47	48	49	50	51	52	53	54	55	56	57	Unscheduled -- Blood Smear Positive (B)
		Study Day	1121	1135	1149	1163	1177	1191	1205	1219	1233	1247	1261	1275	1289	N/A
		Days post-PfSPZ Vaccine	1120	1134	1148	1162	1176	1190	1204	1218	1232	1246	1260	1274	1288	N/A
		Weeks post-PfSPZ Vac #1	160	162	164	166	168	170	172	174	176	178	180	182	184	N/A
		Visit windows (days)	±56	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	
			Screen for Extension/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	Malaria F/U	Unscheduled Visit Positive BS
CLINICAL PROCEDURES																
Informed consent/ Addendum Consent			X													
Focused clinical evaluation (if clinically indicated) (A)			X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment (if indicated)			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review (if indicated)			X	X	X	X	X	X	X	X	X	X	X	X	X	X
LABORATORY PROCEDURES	Designated Laboratory	Tube Type														
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
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	
Research Assays																
Humoral Assays	MRTC/ LMIV	SST	2.5												2.5	
Cellular Assays		NaHep	10.0												10	
Ex Vivo Assays		EDTA	0.5												0.5	0.5
Parasite Purification (CF11)		EDTA														4
Daily total			14	1	1	1	1	1	1	1	1	1	1	1	14	5
Study cumulative total (Extension)			14	15	16	17	18	19	20	21	22	23	24	25	39	

(A) Given >3 year since study product, clinical exam including vital signs will only be completed if clinically indicated.

(B) For unscheduled blood smear positive visits -- for first positive blood smear, subjects should return within 48 hours to blood draw; no need to repeat blood smear given positive BS prompting visit unless clinically indicated; first positive BS = first positive post start of Year 2 or new positive BS occurring >14 days after recent anti-malarial treatment or new positive BS occurring >28 days after negative BS regardless of treatment; visit is optional, with priority for completing visit for first positive BS post start of Year #2

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 10.3.1**

***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 (Female) -- gm/dL	8.00 – 9.00	7.00 – 7.99	6.00 – 6.99	< 6.00 and /or requiring transfusion
WBC Increase – 10^3/µL	11.50 – 15.09	15.10 – 20.09	20.10 – 25.00	> 25.00
WBC Decrease - 10^3/µL	2.50 – 3.30	1.50 – 2.49	1.00 – 1.49	< 1.00 with fever
Granulocyte or Neutrophil Decrease - 10^3/µL	0.80 – 1.00	0.50 – 0.79	< 0.50	< 0.50 with fever
Platelets Decreased – 10^3/µL	100.0 – 110.0	70.0 – 99.9	25.0 – 69.9	< 25.0
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
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 (Infant) - gm/dL	
0-30 days	15.0-22.0
1 month	10.5-14.0
2-6 months	9.5-13.5
≥7 months	10.5-14.0
WBC – 10^3/µL	3.6 – 9.0
Absolute Granulocyte or Neutrophil Count - 10^3/µL	1.3 – 4.4
Absolute Lymphocyte Count - 10^3/µL	1.3 – 4.4
Platelet Count (Female)- 10^3/µL	144 – 413

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old) and infant parameters collection from healthy Oulessebougou infants.

Toxicity Tables for Neonates/Infants (referenced from Munoz et al. 2014)(Munoz, Weisman et al. 2014)

Event Definition Normal Range	Assessment of Severity Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Assessment of neonatal and infant adverse events					
Preterm birth[a] Based on gestational age assessed by best available obstetric estimate, usually prenatal ultrasound or last menstrual period if ultrasound not available	Born at or after 37 wk gestation	Late preterm: 34 to <37 wk gestation	Preterm: 32 to <34 wk gestation	Very preterm: 28 to <32 wk gestation	Extreme preterm: <28 wk
BW, g	BW >2500–3999 Varies with gestational age, sex, race, ethnicity, maternal BMI, and other maternal health factors	NA	Low BW: 1501–2500 High BW : ≥4000 g associated with infant morbidity requiring medical intervention	Very low BW: 1001–1500 High BW: ≥4000 g associated with infant morbidity resulting in prolonged hospitalization	Extremely low BW: ≤1000
Birth weight in relation to gestational age (based on best obstetric estimate, usually prenatal ultrasound or last menstrual period if ultrasound not available)	Based on population specific curves	NA	SGA: BW <10% for infants of same gestational age in same population LGA: >90% for infants of same gestational age in same population	SGA associated with morbidity resulting in prolonged hospitalization LGA associated with infant morbidity resulting in prolonged hospitalization	NA
Birth length	Varies with gestational age, sex, race, ethnicity, and parental height	NA	<10% for gestational age	<1% for gestational age	NA
Birth FOC Microcephaly, low FOC Macrocephaly, high FOC	Varies with gestational age, sex, race and ethnicity	NA	Low FOC: >2–3 SD below mean for gestational age and sex High FOC: >2–3 SD above mean for gestational age and sex	Low FOC: >3 SD below mean for gestational age and sex High FOC: >3 SD above mean for gestational age and sex	NA
General assessment					
Neonatal complications in a term infant, including congenital anomalies[b], clinical or laboratory abnormalities, and events not listed in this table	Normal term infant discharged home with mother after uncomplicated delivery and nursery course	Transient or minimal signs, symptoms, or findings requiring no intervention, symptomatic treatment, or only monitoring, and resolved at the time of discharge	Signs, symptoms or findings requiring intervention or specific therapy resulting in prompt clinical response or resolution and infant discharged home within a week of initiation of therapy	Signs, symptoms, or findings requiring therapy and/or interventions (including surgery) leading to prolonged hospitalization	Life-threatening laboratory or/and clinical signs and symptoms
Systemic conditions					
Fever Elevated body temperature assessed by axillary temperature	Normal newborn temperature: 36.5°C–37.6°C (97.7°F–99.7°F)	37.7°C–38.6°C (99.8°F–101.4°F) resolving spontaneously or with environmental measures (eg, removal of clothing, blankets or heat source)	38.7°C–39.3°C (101.5°F–102.7°F) transient, with or without symptoms, or requiring medical treatment	39.4°C–40.5°C (102.8°F–104.9°F) with associated clinical symptoms, or requiring medical treatment	>40.5°C (>104.9°F), and/or shock[c], convulsions, coma

Event Definition Normal Range	Assessment of Severity				
	Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Hypothermia[d]: Low body temperature, assessed by axillary temperature Preterm and low-BW infants are at risk Symptoms may include: Decreased activity, weak cry, decreased feeding ability, cool skin	Normal newborn temperature: 36.5°C–37.6°C (97.7°F–99.7°F) Affected by gestational age, environment at delivery and postnatally, and neonatal conditions.	36.0°C to <36.5°C (96.8°F to <97.7°F), resolving with routine measures to maintain normal temperature after birth[d]	<36.0°C–35.0°C (<96.8°F–95.0°F) requiring and responding to intervention needed in addition to routine measures to maintain normal temperature	<36.0°C–35.0°C (<98.9°F–95.0°F) persistent despite intervention	<35.0°C (<95.0°F)
Infection (congenital or acquired)	None	Localized, superficial, self-limited, or requiring only topical or oral therapy	Localized or systemic, requiring evaluation and systemic treatment with adequate response	Systemic, single or multiorgan involvement, requiring prolonged therapy. May result in long term sequelae	Sepsis, shock Life-threatening congenital infection
Bleeding Symptoms may include tachycardia, hypotension, diaphoresis, lethargy, pallor, cyanosis, shock	None	Transient, low volume, not associated with symptoms and not requiring intervention	Symptomatic, responsive to volume replacement	Symptomatic, resulting in anemia, requiring transfusion	Shock, anemia, bleeding requiring multiple transfusions
Failure to thrive or growth deficiency Failure to grow and develop normally compared to infants of the same gestational and postnatal age; or inability to maintain expected growth rate over time, evaluated by plotting individual weight gain and growth on standard growth charts for the population	Normal newborn weight gain includes weight loss of up to 10% of birth weight in the first 1–2 weeks of life, with steady, predictable weight gain thereafter. Progress varies by gestational and postnatal age, genetic, and environmental factors	Growth is slow or stopped progressing Requires dietary supplementation to maintain weight gain	Weight for age below the 5th percentile for age, or <80% ideal body weight for age, and/or requires alternative methods of enteral nutrition (nasogastric or nasoduodenal or gastric tube feedings) to maintain weight gain	Weight for age below the 5th percentile for age, or <80% ideal body weight for age, and/or requires parenteral nutrition or surgical interventions to maintain weight gain	NA
Respiratory					
Respiratory distress Assessed by evaluation of RR, nasal flaring, grunting, retractions, pallor, and cyanosis or hypoxemia.	Unlabored breathing and RR, no oxygen requirement RR varies with gestational age and decreases with postnatal age. A term newborn normal RR is usually 30–60 breaths/min	Transient tachypnea and/or hypoxemia (oxygen saturation <95%) requiring brief period of oxygen supplementation with <70% FiO_2	Persistent tachypnea and/or hypoxemia requiring high (70%–100%) FiO_2 supplementation or CPAP. Associated with other clinical symptoms (nasal flaring, grunting, retractions, pallor, or cyanosis)	Respiratory failure requiring mechanical ventilation and or inhaled nitric oxide	Respiratory failure requiring high frequency oscillatory ventilation or extracorporeal membrane oxygenation

Event Definition Normal Range	Assessment of Severity Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Apnea[e] Cessation of breathing for 15 s or more, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor, and/or hypotonia	None	Transient, requires no intervention except stimulation and/or airway clearing	Frequent, recurring, prolonged, and/or requiring oxygen supplementation or medication	Frequent, recurring, prolonged and/or requiring CPAP and/ or medication	Requires intubation for ventilatory support Acute life-threatening event: combination of apnea, color change (cyanosis, pallor, or plethora), marked limpness, and choking or gagging
Cardiovascular					
Hypotension	Blood pressure varies by gestational age and sex	Transient asymptomatic decrease in systolic or mean arterial blood pressure at least 10 mm Hg below normal for age and sex	Symptomatic and persistent decrease in systolic or mean arterial blood pressure at least 15 mm Hg below normal for age and sex	Symptomatic and persistent decrease in systolic or mean arterial blood pressure ≥20 mm Hg below normal for age and sex	Shock, end organ failure (particularly renal), ischemic injury as a result of hypotension
Hypertension	Blood pressure varies by gestational age and sex, and length/height	Transient asymptomatic increase in systolic or mean arterial blood pressure at least 25 mm Hg above normal for age and sex	Symptomatic increase in systolic or mean arterial blood pressure at least 25 mm Hg above normal for age and sex OR in the 91st–94th percentile for age, length, and sex (systolic and/or diastolic)	Symptomatic increase in systolic or mean arterial blood pressure at least 30 mm Hg above normal for age and sex OR >95th percentile for age, length, and gender (systolic and/or diastolic)	Shock, end organ failure (particularly heart and renal), intracranial or retinal hemorrhage, other hypertension related sequelae
Tachycardia: Heart rate above normal range for gestational age and postnatal age Bradycardia: Heart rate below normal range for gestational age and postnatal age	Heart rate within normal ranges for gestational and postnatal age	Transient, asymptomatic increase or decrease in heart rate, not requiring intervention	Asymptomatic or symptomatic increase or decrease in heart rate, responsive to medical therapy	Symptomatic increase or decrease in heart rate, requiring urgent and/or prolonged medical therapy	Persistent increase or decrease in heart rate despite medical therapy, cardiogenic shock
Heart failure	None	Cardiac dysfunction documented by ECG and/or echocardiography, not requiring intervention	Cardiac dysfunction requiring nonurgent medical therapy	Symptomatic, non-life-threatening cardiac dysfunction requiring medical therapy	Symptomatic, life-threatening cardiac dysfunction requiring medical therapy and mechanical ventilation support
Neurologic					
Mental status	Varies with infant gestational age and postnatal age	Transient lethargy or irritability	Persistent lethargy or irritability requiring intervention	Unresponsiveness, lethargy or irritability associated with event that could result in long-term sequelae	Non–medically induced coma that results in failure of spontaneous respirations

Event Definition Normal Range	Assessment of Severity				
	Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Seizures	None observed or suspected	Brief (<5 min), nonfocal, resolved spontaneously or upon correction of precipitating factor, not requiring anticonvulsant therapy	Focal or generalized, recurrent or persistent, lasting 5–20 min with <24 h. postictal state, receiving anticonvulsant therapy	Focal or generalized, recurrent or persistent, lasting >20 min, prolonged postictal period, receiving anticonvulsant therapy	Status epilepticus. Refractory seizures not responding to treatment
Muscle tone and reflexes	Normal for gestational and postnatal age	Transiently decreased or increased, not requiring specific therapy	Persistently decreased or increased requiring evaluation and specific therapy	Persistently decreased or increased, requiring additional testing and specific therapy, not improving	Neuromuscular disease that is incompatible with life
Developmental delay	Normal development for gestational and postnatal age based on history, physical examination, and standard assessment tools	Mild delay in motor or cognitive skills as determined by developmental screening tool appropriate for age and setting	Moderate delay in motor or cognitive skills as determined by developmental screening tool appropriate for age and setting	Severe delay or regression in motor or cognitive skills as determined by developmental screening tool appropriate for age and setting	NA
Musculoskeletal					
Arthritis: stiffness or joint swelling, with or without erythema, usually associated with pain Myositis: muscle swelling or induration, with or without erythema, usually associated with pain and limited mobility		Transient and mild arthritis or myositis <24 h in duration	Transient arthritis or myositis 24–72 h duration	Persistent, disabling myositis or arthritis, with or without systemic signs	NA
Gastrointestinal					
Difficulty feeding	None	Transient difficulty feeding not resulting in additional intervention	Difficulty feeding resulting in need for supplemental or alternative methods of feeding, resolves with specific therapy	Difficulty feeding requiring intervention, including medications, parenteral nutrition, and/or surgery and affecting infant growth	Difficulty feeding requiring intervention, including medications, parenteral nutrition, and/or surgery and affecting infant growth, does not resolve or not expected to resolve over time
Vomiting	None	Postfeeding, small volume, occasional, not altering feeds, no dehydration	May or may not be associated with feeds, frequent, requiring evaluation and/or treatment, but not affecting growth or hydration status	Persistent vomiting, requiring treatment and intravenous fluids; only transiently affects growth or hydration status	Vomiting associated with hypotension and/or shock, or poor growth despite intervention

Event Definition Normal Range	Assessment of Severity				
	Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Diarrhea	Newborn stools vary depending on type of feeding, formula vs breast milk	Transient change in consistency (liquid stools) but not frequency of stools, normal feeding, no dehydration, no intervention	Change in consistency (liquid stools) and/or frequency of stools, requiring intervention to prevent or treat dehydration with prompt resolution	Persistent diarrhea requiring treatment for dehydration and/or specific etiologic treatment	Diarrhea associated with hypotension and shock, requiring aggressive rehydration
Liver dysfunction evaluated by measurement of liver enzymes (AST, ALT, GGT, alkaline phosphatase) Each value graded independently	Values vary by gestational and postnatal age	1.25 to <2.5 × ULN	2.5 to <5 × ULN	5 × to <10 × ULN	≥10 × ULN requiring surgical intervention or liver transplant
Hyperbilirubinemia[f]: total bilirubin (mg/dL)[g] (not cholestasis related)	Normal levels vary by gestational and postnatal age and change in the first days and weeks of life, also affected by breastfeeding status	<1.5 × ULN for age and gestational age	1.6–2.0 × ULN	2.1–5.0 × ULN or reach cutoff for indication of phototherapy	>5.1 × ULN or reach cutoff for indication of exchange transfusion
Hyperbilirubinemia[f]: direct bilirubin (mg/dL)[g] (cholestasis)	Normal levels vary by gestational and postnatal age and change in the first days and weeks of life, also affected by breastfeeding status	1.0–1.5 mg/dL	1.6–2.0 mg/dL	2.1–2.5 mg/dL OR >ULN and >10% of total bilirubin	>2.6 mg/dL OR >ULN with signs and symptoms of liver failure
Hematologic					
Anemia[g] (hemoglobin in mg/dL)	Hemoglobin and hematocrit levels vary by gestational age, sex, and race/ethnicity	<7 d 13.0–14.0 7–60 d 8.5–9.4	<7 d 12.0–12.9 7–60 d 7.0–8.4	<7 d <12.0 7–60 d <7.0 OR any value requiring transfusion	Congestive heart failure due to anemia
Leukopenia[g]: WBC (cells/mm^3) decreased	Varies by gestational age, sex, and race/ethnicity	2000 to <2500	1500 to <2000	1000 to <1500	<1000
Neutropenia[g] ANC (cells/mm^3) decreased	Varies by gestational age, sex, and race/ethnicity	≤1 d: 4000 to <5000 2–7 d: ANC 1250 to <1500 7–60 d: ANC 1000 to <1300	≤1 d: 3000 to <4000 2–7 d: ANC 1000 to <1250 7–60 d: ANC 750 to <1000	≤1 d 1500 to <3000 2–7 d: ANC 750 to <1000 7–60 d: ANC 500 to <750	≤1 d <1500 2–7 d: ANC <750 7–60 d: ANC <500
Thrombocytopenia[h]: Platelet count (platelets/mm^3) decreased	Normal varies with gestational and postnatal age, usually ≥150 000	100 000–149 000	50 000 to <100 000	25 000 to <50 000	<25 000 OR any value associated with bleeding

Event Definition Normal Range	Assessment of Severity				
	Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Coagulopathy[h]: INR of prothrombin time	Normal range varies with gestational and postnatal age, usually 0.9–1.1	<1.5 × ULN	1.5 to <2.0 × ULN	2.0 to <3.0 × ULN	≥3.0 × ULN
Bruising, petechiae, or ecchymosis	None	Localized, self-limited	Localized or widespread covering <50% of body surface	Covering ≥50% of body surface	NA
Metabolic/endocrine					
Hypoglycemia[i]: Low serum glucose (mg/dL) Represents failure to adapt from fetal continuous transplacental source of glucose to postnatal nutrient supply, or a nonspecific sign of illness	Normal range not defined. Values vary with birth weight, gestational and postnatal age, and feeding method. Preterm and SGA infants are at risk.	Transiently low <50 resolving with feeds	Transiently or persistently low, <50 not responding to oral feeds and requiring intravenous bolus of dextrose	Persistently low, <50 or symptomatic (apnea, cyanosis, jitteriness), unresponsive to bolus therapy and/or requiring continuous intravenous dextrose infusion and/or steroids	Hypoglycemia <50 associated with seizures, respiratory failure or cardiac arrhythmia
Calcium[g] (mg/dL) Hypocalcemia (low serum calcium): <8 hypercalcemia (high serum calcium): >11.0	Values vary in first days of life and with gestational and postnatal age	<7 d: Low: 6.5 to <7.5 High: 11.5 to <12.5 7–60 d: Low: 7.8 to <8.4 High: 10.6 to <11.5	<7 d: Low: 6.0 to <6.5 High: 12.5 to <13.0 7–60 d: Low: 7.0 to <7.8 High: 11.5 to <12.5	<7 d: Low: 5.5 to <6.0 High: 13.0 to <13.5 7–60 d: Low: 6.1 to <7.0 High: 12.5 to <13.5	<7 d: Low: <5.5 High: ≥13.5 7–6 d: Low: <6.1 High: ≥13.5
Ionized calcium[g] (mmol/L)		Low: ≤LLN to >1.0 High: ≥ULN to <1.5	Low: ≤1.0 to >0.9 and symptomatic; High: ≥1.5 to <1.6 and symptomatic	Low: ≤0.9 to >0.8 symptomatic, treatment indicated High: ≥1.6 to <1.8 symptomatic, treatment indicated	Low: ≤0.8 with life-threatening consequences High: ≥1.8 with life-threatening consequences
Magnesium[g] (meq/L)	Values vary in first days of life	Low: 1.2 to <1.4	0.9 to <1.2	0.6 to <0.9	<0.6
Renal					
Renal insufficiency: CrCl (mL/min/1.73 m^2) decreased	CrCl varies with gestational age and postnatal age	Normal urinary output and mild elevation of serum creatinine	CrCl <LLN to 60	CrCl <60 to ≥30 or requiring renal replacement therapy	CrCl <30 requiring any form of renal replacement therapy
Creatinine[g] (mg/dL) elevation may be associated with renal insufficiency	Values vary in first days of life and with gestational age; might reflect maternal creatinine	<7 d: 1.0–1.7 7–60 d: 0.5–0.9 OR 1.1–1.2 × ULN	<7 d: 1.8–2.4 7–60 d: 1.0–1.4 OR 1.3–1.7 × ULN	<7 d: 2.5–3.0 7–60 d: 1.5–2.0 OR 1.8–3.3 × ULN	<7 d: >3.0 7–60 d: >2.0 OR >3.4 × ULN
Sodium (meq/L)	Normal values vary with gestational and postnatal age	Low: 126–131 High: 145–149	Low: 121–125 High: 150–154	Low: 116–120 High: 155–164	Low: ≤115 High: ≥165
Potassium (meq/L)	Normal values vary with gestational and postnatal age	Low: 2.8–3.1 High: 5.6–6.1	Low: 2.4–2.7 High: 6.2–6.8	Low: 2.0–2.3 High: 6.9–7.5	Low: <2.0 High: >7.5

Event Definition Normal Range	Assessment of Severity				
	Normal	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life Threatening)
Dermatologic					
Rash		Localized, superficial, resolves promptly with topical treatment	Localized or disseminated, requires evaluation and specific topical and /or systemic treatment	Disseminated, persistent despite specific and/or systemic treatment	Anaphylactic reaction, Stevens-Johnson syndrome, other congenital dermatologic disorders

Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BMI, body mass index; BW, birth weight; CPAP, continuous positive airway pressure; CrCl, creatinine clearance; ECG, electrocardiography; FiO_2, inspired fraction of oxygen; FOC, fronto-occipital head circumference; GGT, γ-glutamyltransferase; INR, international normalized ratio; LGA, large for gestational age; LLN, lower limit of normal; meq/L, milliequivalents per liter; NA, not applicable; RR, respiratory rate; SD, standard deviation; SGA, small for gestational age; ULN, upper limit of normal; WBC, white blood cell.

[a] Preterm birth and low birth weight carry different risks and should be reported separately (or twice if an infant is both preterm and low birth weight). If gestational age is not known, birth weight should be reported. Infants should be plotted in appropriate growth scales for the population being studied and reported as appropriate, large, or small for gestational age [20–22].

[b] Congenital anomalies can be classified as major or minor; severity depends on type of anomaly [23].

[c] Shock is defined as failure of the circulatory system to maintain adequate perfusion of vital organs.

[d] Routine measures include immediate drying, stimulation, appropriate clothing and bedding, skin-to-skin contact with mother, environmental thermal support [24].

[e] Source data can be found in Ref. [25].

[f] Hyperbilirubinemia is measured by total and direct bilirubin levels, which vary substantially according to gestational age at birth and in the first few days of life and the early postnatal period due to physiologic anemia, and is affected by breastfeeding and the function of the gastrointestinal tract. In general, severe hyperbilirubinemia should be consistent with cutoffs for indication for phototherapy, and grade 4 or life-threatening hyperbilirubinemia, with cutoffs for exchange transfusion [26].

[g] Reference and values for age subgroups (<7 days, 7–60 days, 61–90 days) in term and preterm infants, and in infants >3 months of age are available for some parameters at in Refs. [15] and [16].

[h] Source data can be found in Ref. [27–29].

[i] Source data can be found in Ref. [30].

Appendix C: References

Agnandji, S. T., Lell, B., Soulanoudjingar, S. S., Fernandes, J. F., Abossolo, B. P., Conzelmann, C., . . . Vansadia, P. (2011). First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med, 365*(20), 1863-1875. doi:10.1056/NEJMoa1102287

Bethell, D. B., Phuong, P. T., Phuong, C. X., Nosten, F., Waller, D., Davis, T. M., . . . White, N. J. (1996). Electrocardiographic monitoring in severe falciparum malaria. *Trans R Soc Trop Med Hyg, 90*(3), 266-269.

Bregani, E. R., Tien, T. V., Cabibbe, M., Figini, G., & Manenti, F. (2004). Holter monitoring in children with severe *Plasmodium falciparum* malaria during i.v. quinine treatment. *J Trop Pediatr, 50*(1), 61.

Campo, J. J., Sacarlal, J., Aponte, J. J., Aide, P., Nhabomba, A. J., Dobano, C., & Alonso, P. L. (2014). Duration of vaccine efficacy against malaria: 5th year of follow-up in children vaccinated with RTS,S/AS02 in Mozambique. *Vaccine, 32*(19), 2209-2216. doi:10.1016/j.vaccine.2014.02.042

Casares, S., & Richie, T. L. (2009). Immune evasion by malaria parasites: a challenge for vaccine development. *Curr Opin Immunol, 21*(3), 321-330. doi:10.1016/j.coi.2009.05.015

Ceesay, S. J., Casals-Pascual, C., Erskine, J., Anya, S. E., Duah, N. O., Fulford, A. J., . . . Conway, D. J. (2008). Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet, 372*(9649), 1545-1554. doi:10.1016/S0140-6736(08)61654-2

Chen, Q., Fernandez, V., Sundstrom, A., Schlichtherle, M., Datta, S., Hagblom, P., & Wahlgren, M. (1998). Developmental selection of var gene expression in Plasmodium falciparum. *Nature, 394*(6691), 392-395. doi:10.1038/28660

Clyde, D. F. (1990). Immunity to falciparum and vivax malaria induced by irradiated sporozoites: a review of the University of Maryland studies, 1971-75. *Bull World Health Organ, 68 Suppl*, 9-12.

Clyde, D. F., McCarthy, V. C., Miller, R. M., & Hornick, R. B. (1973). Specificity of protection of man immunized against sporozoite-induced falciparum malaria. *Am J Med Sci, 266*(6), 398-403.

Das, P., & Horton, R. (2010). Malaria elimination: worthy, challenging, and just possible. *Lancet, 376*(9752), 1515-1517. doi:10.1016/S0140-6736(10)61551-6

Diallo, D. A., Doumbo, O. K., Plowe, C. V., Wellems, T. E., Emanuel, E. J., & Hurst, S. A. (2005). Community permission for medical research in developing countries. *Clin Infect Dis, 41*(2), 255-259. doi:10.1086/430707

Dicko, A., Diallo, A. I., Tembine, I., Dicko, Y., Dara, N., Sidibe, Y., . . . Greenwood, B. (2011). Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Mali: a randomised, double-blind, placebo-controlled trial. *PLoS Med, 8*(2), e1000407. doi:10.1371/journal.pmed.1000407

Ehrhardt, S., Mockenhaupt, F. P., Anemana, S. D., Otchwemah, R. N., Wichmann, D., Cramer, J. P., . . . Brattig, N. W. (2005). High levels of circulating cardiac proteins indicate cardiac impairment in African children with severe *Plasmodium falciparum* malaria. *Microbes Infect, 7*(11-12), 1204-1210. doi:S1286-4579(05)00144-9 [pii]

10.1016/j.micinf.2005.04.007

Epstein, J. E., Paolino, K. M., Richie, T. L., Sedegah, M., Singer, A., Ruben, A. J., . . . Hoffman, S. L. (2017). Protection against Plasmodium falciparum malaria by PfSPZ Vaccine. *JCI Insight, 2*(1), e89154. doi:10.1172/jci.insight.89154

Epstein, J. E., Tewari, K., Lyke, K. E., Sim, B. K., Billingsley, P. F., Laurens, M. B., . . . Hoffman, S. L. (2011). Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. *Science, 334*(6055), 475-480. doi:10.1126/science.1211548

Grabowsky, M. (2008). The billion-dollar malaria moment. *Nature, 451*(7182), 1051-1052. doi:10.1038/4511051a

Hermsen, C. C., Telgt, D. S., Linders, E. H., van de Locht, L. A., Eling, W. M., Mensink, E. J., & Sauerwein, R. W. (2001). Detection of *Plasmodium falciparum* malaria parasites in vivo by real-time quantitative PCR. *Mol Biochem Parasitol, 118*(2), 247-251. doi:S0166685101003796 [pii]

Hernan, M. A. (2010). The hazards of hazard ratios. *Epidemiology, 21*(1), 13-15. doi:10.1097/EDE.0b013e3181c1ea43

Hoffman, S. L., Billingsley, P. F., James, E., Richman, A., Loyevsky, M., Li, T., . . . Sim, B. K. (2010). Development of a metabolically active, non-replicating sporozoite vaccine to prevent Plasmodium falciparum malaria. *Hum Vaccin, 6*(1), 97-106.

Hoffman, S. L., Goh, L. M., Luke, T. C., Schneider, I., Le, T. P., Doolan, D. L., . . . Richie, T. L. (2002). Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. *J Infect Dis, 185*(8), 1155-1164. doi:10.1086/339409

Howie, S. R. (2011). Blood sample volumes in child health research: review of safe limits. *Bull World Health Organ, 89*(1), 46-53. doi:10.2471/BLT.10.080010

Ishizuka, A. S., Lyke, K. E., DeZure, A., Berry, A. A., Richie, T. L., Mendoza, F. H., . . . Seder, R. A. (2016). Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nature Medicine, 22*(6), 614-623. doi:nm.4110 [pii]

10.1038/nm.4110

Korenromp, E. L., Williams, B. G., Gouws, E., Dye, C., & Snow, R. W. (2003). Measurement of trends in childhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy. *Lancet Infect Dis, 3*(6), 349-358.

Lyke, K. E., Laurens, M., Adams, M., Billingsley, P. F., Richman, A., Loyevsky, M., . . . Hoffman, S. L. (2010). Plasmodium falciparum malaria challenge by the bite of aseptic Anopheles stephensi mosquitoes: results of a randomized infectivity trial. *PLoS One, 5*(10), e13490. doi:10.1371/journal.pone.0013490

Mellouk, S., Lunel, F., Sedegah, M., Beaudoin, R. L., & Druilhe, P. (1990). Protection against malaria induced by irradiated sporozoites. *Lancet, 335*(8691), 721.

Murray, C. J., Rosenfeld, L. C., Lim, S. S., Andrews, K. G., Foreman, K. J., Haring, D., . . . Lopez, A. D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet, 379*(9814), 413-431. doi:10.1016/S0140-6736(12)60034-8

Ndugwa, R. P., Ramroth, H., Muller, O., Jasseh, M., Sie, A., Kouyate, B., . . . Becher, H. (2008). Comparison of all-cause and malaria-specific mortality from two West African countries with different malaria transmission patterns. *Malar J, 7*, 15. doi:10.1186/1475-2875-7-15

Nganou-Makamdop, K., Ploemen, I., Behet, M., Van Gemert, G. J., Hermsen, C., Roestenberg, M., & Sauerwein, R. W. (2012). Reduced Plasmodium berghei sporozoite liver load

associates with low protective efficacy after intradermal immunization. *Parasite Immunol, 34*(12), 562-569. doi:10.1111/pim.12000.x

Nieman, A. E., de Mast, Q., Roestenberg, M., Wiersma, J., Pop, G., Stalenhoef, A., . . . van der Ven, A. (2009). Cardiac complication after experimental human malaria infection: a case report. *Malar J, 8*, 277. doi:1475-2875-8-277 [pii]

10.1186/1475-2875-8-277

Nussenzweig, R. S., Vanderberg, J., Most, H., & Orton, C. (1967). Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. *Nature, 216*(5111), 160-162.

O'Meara, W. P., Bejon, P., Mwangi, T. W., Okiro, E. A., Peshu, N., Snow, R. W., . . . Marsh, K. (2008). Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet, 372*(9649), 1555-1562. doi:10.1016/S0140-6736(08)61655-4

O'Meara, W. P., Mwangi, T. W., Williams, T. N., McKenzie, F. E., Snow, R. W., & Marsh, K. (2008). Relationship between exposure, clinical malaria, and age in an area of changing transmission intensity. *Am J Trop Med Hyg, 79*(2), 185-191.

Plebanski, M., Flanagan, K. L., Lee, E. A., Reece, W. H., Hart, K., Gelder, C., . . . Hill, A. V. (1999). Interleukin 10-mediated immunosuppression by a variant CD4 T cell epitope of Plasmodium falciparum. *Immunity, 10*(6), 651-660.

Ploemen, I. H., Chakravarty, S., van Gemert, G. J., Annoura, T., Khan, S. M., Janse, C. J., . . . Sauerwein, R. W. (2013). Plasmodium liver load following parenteral sporozoite administration in rodents. *Vaccine, 31*(34), 3410-3416. doi:10.1016/j.vaccine.2012.09.080

Rieckmann, K. H., Carson, P. E., Beaudoin, R. L., Cassells, J. S., & Sell, K. W. (1974). Letter: Sporozoite induced immunity in man against an Ethiopian strain of Plasmodium falciparum. *Trans R Soc Trop Med Hyg, 68*(3), 258-259.

Roberts, L., & Enserink, M. (2007). Malaria. Did they really say ... eradication? *Science, 318*(5856), 1544-1545. doi:10.1126/science.318.5856.1544

Rts, S. C. T. P. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet*. doi:10.1016/S0140-6736(15)60721-8

Schneider, P., Schoone, G., Schallig, H., Verhage, D., Telgt, D., Eling, W., & Sauerwein, R. (2004). Quantification of *Plasmodium falciparum* gametocytes in differential stages of development by quantitative nucleic acid sequence-based amplification. *Mol Biochem Parasitol, 137*(1), 35-41. doi:10.1016/j.molbiopara.2004.03.018

S0166685104001379 [pii]

Seder, R. A., Chang, L. J., Enama, M. E., Zephir, K. L., Sarwar, U. N., Gordon, I. J., . . . Team, V. R. C. S. (2013). Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science, 341*(6152), 1359-1365. doi:10.1126/science.1241800

Sissoko, M. S., Healy, S. A., Katile, A., Omaswa, F., Zaidi, I., Gabriel, E. E., . . . Duffy, P. E. (2017). Safety and efficacy of PfSPZ Vaccine against Plasmodium falciparum via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial. *Lancet Infect Dis, 17*(5), 498-509. doi:10.1016/S1473-3099(17)30104-4

Stoute, J. A., Kester, K. E., Krzych, U., Wellde, B. T., Hall, T., White, K., . . . Marchand, M. (1998). Long-term efficacy and immune responses following immunization with the RTS,S malaria vaccine

A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparum malaria. RTS,S Malaria Vaccine Evaluation Group. *J Infect Dis, 178*(4), 1139-1144. doi:10.1056/NEJM199701093360202

Urban, B. C., Ferguson, D. J., Pain, A., Willcox, N., Plebanski, M., Austyn, J. M., & Roberts, D. J. (1999). Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells. *Nature, 400*(6739), 73-77. doi:10.1038/21900

van Meer, M. P., Bastiaens, G. J., Boulaksil, M., de Mast, Q., Gunasekera, A., Hoffman, S. L., . . . Sauerwein, R. W. (2014). Idiopathic acute myocarditis during treatment for controlled human malaria infection: a case report. *Malar J, 13*, 38. doi:10.1186/1475-2875-13-38

Walther, B., & Walther, M. (2007). What does it take to control malaria? *Ann Trop Med Parasitol, 101*(8), 657-672. doi:10.1179/136485907X229013

Walther, M. (2006). Advances in vaccine development against the pre-erythrocytic stage of Plasmodium falciparum malaria. *Expert Rev Vaccines, 5*(1), 81-93. doi:10.1586/14760584.5.1.81

Walther, M., Dunachie, S., Keating, S., Vuola, J. M., Berthoud, T., Schmidt, A., . . . Hill, A. V. (2005). Safety, immunogenicity and efficacy of a pre-erythrocytic malaria candidate vaccine, ICC-1132 formulated in Seppic ISA 720. *Vaccine, 23*(7), 857-864. doi:S0264-410X(04)00642-5 [pii]

10.1016/j.vaccine.2004.08.020

Walther, M., Thompson, F. M., Dunachie, S., Keating, S., Todryk, S., Berthoud, T., . . . Hill, A. V. (2006). Safety, immunogenicity, and efficacy of prime-boost immunization with recombinant poxvirus FP9 and modified vaccinia virus Ankara encoding the full-length *Plasmodium falciparum* circumsporozoite protein. *Infect Immun, 74*(5), 2706-2716. doi:74/5/2706 [pii]

10.1128/IAI.74.5.2706-2716.2006

WHO. (2017). World Health Malaria Report.

World malaria report 2011. (2011). Geneva Retrieved from http://www.who.int/malaria/world_malaria_report_2011/en/.