Sanaria® PfSPZ Challenge with Pyrimethamine or Chloroquine Chemoprophylaxis Vaccination (PfSPZ-CVac Approach): A Randomized Double Blind Placebo Controlled Phase I/II Trial to Determine Safety and Protective Efficacy against Natural *Plasmodium falciparum* Infection in Bancoumana and Surrounding Areas, Mali

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Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)
Laboratory of Malaria Immunology and Vaccinology (LMIV)
National Institutes of Health (NIH)

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Sanaria Inc.
IND: 16650

Clinical Trial Monitoring Delegated to NIAID DCR Office of Clinical Research Policy and Regulatory Operations

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List of Abbreviations

AE	adverse event
AES	asexual erythrocytic stages
AGC	absolute granulocyte count
ALT	alanine transaminase
AMA-1	Apical membrane antigen -1
ANC	absolute neutrophil count
AR	adverse reaction
AST	aspartate aminotransferase
β-hCG	β human choriogonadotropin
CBC	complete blood count
CFR	Code of Federal Regulations
CHI	Center for Human Immunology, Autoimmunity and Inflammation
cGMP	current Good Manufacturing Practices
CHMI	controlled human malaria infection
CoA	Certificate of Analysis
CPS	Chemoprophylaxis with sporozoites
CQ	chloroquine phosphate
Cr	creatinine
CRF	case report form
CSP	circumsporozoite protein
CVac	chemoprophylaxis vaccination
DMID	Division of microbiology and infectious diseases
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
DVI	Direct Venous Inoculation
EBA-175	erythrocyte binding antigen 175
EC	ethics committee
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot assay
EXP-1	exported protein 1
FDA	Food and Drug Administration
FMPOS	Faculty of Medicine and Odonto-Stomatology
GCP	good clinical practice
Hb	hemoglobin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HSA	human serum albumin

IB	investigator's brochure
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
ID	intradermal
IFA	immunofluorescence assay
IM	intramuscular
IND	Investigational New Drug
IPTp	intermittent preventive treatment in pregnancy
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITV	Infection treatment vaccination
IV	intravenous
LDH	lactate dehydrogenase
LMIV	Laboratory of Malaria Immunology and Vaccinology (of NIAID)
LNVP	liquid nitrogen vapor phase
LSA-1	liver stage antigen 1
MCB	master cell bank
MRTC	Malaria Research and Training Center
MSP-1	merozoite surface protein 1
MSP-5	merozoite surface protein 5
N	number (typically refers to subjects or participants)
NASBA	nucleic acid sequence–based amplification
NHMI	Natural Human Malaria Infection
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NOCI	new onset of chronic illness
NS	normal saline
NSAID	non-steroidal anti-inflammatory drug, e.g., ibuprofen, naproxen
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSRP	Office of Human Subjects Research Protections
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PI	principal investigator
Pf	*Plasmodium falciparum*
Pf7G8	*Plasmodium falciparum, 7G8 clone*
PfNF54	*Plasmodium falciparum, NF54 strain*
PfAES	*Plasmodium falciparum* asexual erythrocytic stages
PfSPZ	*Plasmodium falciparum* sporozoites
PfSPZ-CVac	PfSPZ challenge chemoprophylaxis vaccination

PfSPZ Challenge	Aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites used for immunization and for controlled human malaria infection (CHMI)
POI	premature ovarian insufficiency
PYR	pyrimethamine
qPCR	quantitative polymerase chain reaction
RUNMC	Radboud University Nijmegen Medical Center
SAE	serious adverse event/serious adverse experience
SAR	suspected adverse reaction
sBS	sensitive blood smears
sc	subcutaneous
SERF	Safety Expedited Report Form
SMC	safety monitoring committee
SOP	standard operating procedure
SPZ	sporozoites
SUSAR	serious and unexpected suspected adverse reaction
UCRC	University Clinical Research Center
UP	unanticipated problem
USTTB	University of Sciences, Techniques and Technologies of Bamako
VE	vaccine efficacy
WBC	white blood cell
WCB	working cell bank
WHO	World Health Organization
WSQ	Wavelet Scalar Quantization

PROTOCOL SUMMARY

Full Title:	Sanaria® PfSPZ Challenge with Pyrimethamine or Chloroquine Chemoprophylaxis Vaccination (PfSPZ-CVac Approach): A Randomized Double Blind Placebo Controlled Phase I/II Trial to Determine Safety and Protective Efficacy against Natural *Plasmodium falciparum* Infection in Bancoumana and Surrounding Areas, Mali
Short Title:	Mali PfSPZ-CVac (pyrimethamine)
Clinical Phase:	I/II
IND Sponsor:	Sanaria Inc.
Clinical Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Conducted by:	Malaria Research and Training Center (MRTC), in collaboration with the Laboratory of Malaria Immunology and Vaccinology (LMIV)
Principal Investigators:	Issaka Sagara, MD, MPH, PhD (MRTC) Patrick Duffy, MD (LMIV/NIAID/NIH)

Site	MRTC (Bancoumana, Mali)
Sample Size:	N=432-440
Accrual Ceiling:	800
Study Population:	Healthy Malian adults, 18-50 years of age
Accrual Period:	Approximately April 2019 to April 2022 (Main Phase) Approximately June 2020 to April 2023 (Booster Phase)
Study Duration:	Start Date: Approximately April 2019 End Date: Approximately April 2023 Study subjects will be enrolled (exclusive of screening) for a total of approximately 1 (pilot) to 22 (main + booster) months
Study Design:	This is a randomized double-blind placebo controlled phase I/II study to investigate the safety, tolerability, and immunogenicity of direct venous inoculation (DVI) with three monthly doses of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites(PfSPZ) (Sanaria® PfSPZ Challenge (NF54)[1]), combined with either pyrimethamine (PYR) or chloroquine as a partner drug, known as PfSPZ Challenge chemoprophylaxis vaccination (Sanaria® PfSPZ-CVac). ***In brief:*** • **Pilot Arms:** to assess whether administering PYR on the same day as PfSPZ Challenge and also increasing the dose of PfSPZ Challenge to $4x10^5$ PfSPZ under either PYR or chloroquine prophylaxis is safe and tolerable o **Pyrimethamine arms** ▪ ***Arm 1a:*** **$4x10^5$** PfSPZ with **75 mg** PYR day 0 post PfSPZ (n=4) ▪ ***Arm 2a:4*** **$x10^5$** PfSPZ with **75 mg** PYR days 2 & 3 post PfSPZ (n=4) ▪ ***Arm 5a*** (if necessary)***: 3*** **$x10^5$** PfSPZ with **75 mg** PYR day 0

[1] Hereafter PfSPZ Challenge (NF54) will be denoted PfSPZ Challenge, unless being compared to another strain or clone. If a PfSPZ Challenge product is composed of a strain or clone of Pf other than NF54 (e.g., clone 7G8), it will be so designated [e.g., PfSPZ Challenge (7G8)].

post PfSPZ (n=4)
        - ***Arm 6a*** (if necessary)***: 3*****x10^5** PfSPZ with **75 mg** PYR day 2&3 post PfSPZ (n=4)
    - **Chloroquine arm (4x10^5 PfSPZ)**
        - ***Arm 3a:*** chloroquine 2 days prior + 5 days post PfSPZ (n=4)
- **Main Arms (n=420):** to assess safety and tolerability of 3 exposures every 28 days of 4x10^5 PfSPZ while under PYR given on either day 0 *{or* (if necessary) 3x10^5 PfSPZ while under PYR given on either day 0 (*Arm 5b)} OR* 4x10^5 PfSPZ while under PYR given on day 2 & 3 (*Arm 2b*) post vaccination {or (if necessary) 3x10^5 PfSPZ while under PYR given day 2 & 3 post vaccination *(Arm 6b)*} OR 4x10^5 PfSPZ while under weekly chloroquine +/- non-steroidal anti-inflammatory drugs (NSAIDs) chemoprophylaxis (*Arm 3b*). In addition, the main study will have placebo control groups that will receive normal saline (NS) injections with **either** PYR given on either day 0 *or* 2 & 3 post injection (*Arm 4a* and *4b* respectively) **or** weekly chloroquine chemoprophylaxis (*Arm 4c*). The study will also assess development of protective efficacy against natural *P. falciparum* infection.

The study is to occur in two parts, a pilot study and a main study. In the PYR pilot arms (n=8-16, n=4 in each pilot arm), the goal will be to assess the safety and tolerability of a PfSPZ dose of 4x10^5 PfSPZ under a single dose of 75 mg of PYR chemoprophylaxis given on day 0 (the same day as PfSPZ injection, *Arm 1a*) or on day 2&3 post PfSPZ injection (*Arm 2a*) . Participants will be randomly enrolled in either *Arm 1a* or *Arm 2a*. If the regimen is safe and prevents patent parasitemia, these regimens will move forward to the main study. If there is patent parasitemia in either *Arm 1a* or *Arm 2a*, the dose of PfSPZ will be decreased to 3x10^5 PfSPZ with the same PYR regimen in *Arms 5a* or *6a* respectively. The pyrimethamine main study will be conducted in the first year of the study.

The chloroquine pilot study will assess safety and tolerability of PfSPZ-CVac (chloroquine) with 4x10^5 PfSPZ under chloroquine chemoprophylaxis at a fixed

time (*Arm 3a*: -2 days, +5 days post PfSPZ injection). If the regimen is tolerable (there are no Grade 3 AE symptoms lasting more than 48 hours despite adequate management that are deemed related to resulting parasitemia post PfSPZ injection), this regimen will move forward to the main study. If the regimen is not tolerable, empiric NSAIDs will be administered at days 7 & 8 post first PfSPZ injection in the main study. In another similar study, addition of NSAIDs has been shown to decrease frequency and severity of symptoms related to parasitemia when administered in this schedule. The chloroquine main study will be conducted in a subsequent year of the study.

The regimens selected in the pilot study will then be further evaluated in the main study which is designed to assess safety, tolerability, immunogenicity, and protective efficacy of PfSPZ-CVac (pyrimethamine) and PfSPZ-CVac (chloroquine) against natural *P.falciparum* infection.

At the completion of the follow up period in the first transmission season, subjects in the main study arms will be allowed to participate in a booster phase that will assess the safety, tolerability, immunogenicity and protective efficacy of a 4th vaccination (booster dose). *Arms 1b, 2b,* and *3b* will each receive a single dose of 4x10^5 PfSPZ Challenge whereas *Arms 4a, 4b,* and *4c* will receive normal saline while under their respective chemoprophylaxis regimens (PYR on day 0 for *Arms 1b/4a*, PYR on days 2 and 3 for *Arms 2b/4b*, and CQ on days -2 and 5 for *Arms 3b/4c*). The booster phase will extend follow-up through the subsequent transmission season.

In Detail:
The pilot phase will be open label.

Pyrimethamine Pilot (n=8-16; 4 per arm)
In the PYR pilot, subjects will undergo immunization with PfSPZ-CVac (pyrimethamine) with one exposure to PfSPZ Challenge with 4x10^5 PfSPZ with PYR dosing on day 0 only (same day, *Arm 1a*) or day 2 & 3 (*Arm 2a)* post PfSPZ Challenge. The goal of the pilot study is to first determine that dosing PYR on the same day as PfSPZ DVI (day 0 post DVI; earlier than previously

tested) and with a higher dose of PfSPZ ($4x10^5$, not previously tested) is safe, and does prevent patent parasitemia. Enrollment in these arms will occur simultaneously with 4 participants in each *Arm*. If there is breakthrough parasitemia in either *Arm*, the dose of PfSPZ will be decreased to $3x10^5$ PfSPZ in the arm with the same regimen of PYR. *Arm 5a* will receive $3x10^5$ PfSPZ with PYR administered on day 0 and *Arm 6a* will receive $3x10^5$ PfSPZ with PYR administered on days 2 &3 post PfSPZ injection. If there is breakthrough parasitemia in either *Arm 5a or Arm 6a*, the study will pause for reassessment. All participants will be treated with standard treatment doses of artemether/lumefantrine approximately 2 weeks prior to PfSPZ DVI and at the end of their enrollment. Participants from the pilot study will not join the main study.

Chloroquine Pilot (n=4)

In the chloroquine pilot study (*Arm 3a*), subjects will undergo immunization with PfSPZ-CVac (chloroquine) with one exposure to PfSPZ Challenge with a chloroquine loading dose administered 2 days before PfSPZ Challenge and a maintenance dose administered 5 days post PfSPZ Challenge. The goal of the pilot study is to evaluate safety and tolerability of administering a dose of $4x10^5$ PfSPZ of PfSPZ Challenge while under chloroquine chemoprophylaxis (this dose has not been previously tested). If this regimen is not tolerable (presence of Grade 3 AE symptoms lasting more than 48 hours despite adequate management that are associated with parasitemia starting from day 7 post DVI), then NSAIDs will be administered on day 7 & 8 post DVI during the first vaccination only in the main study). All participants will be treated with standard treatment doses of artemether/lumefantrine approximately 2 weeks prior to PfSPZ DVI and at the end of their enrollment. Participants from the pilot study will not join the main study.

Main Study (n=420)

The main study will be a double-blind study, both investigators and participants will not be aware of the injection assignment (PfSPZ Challenge or normal saline), but they will be aware of the partner drug being administered.

Vaccinated participants will be randomly enrolled in either *Arm 1b* ($4x10^5$ PfSPZ + 75 mg PYR on days 0 post DVI, n=90); *Arm 2b* ($4x10^5$PfSPZ + 75 mg PYR on days 2 & 3 post DVI, n=60); or in *Arm 3b* ($4x10^5$ PfSPZ + weekly chloroquine prophylaxis +/- NSAIDs, n=90).
The control Arm (*Arm 4*, n=180) will receive NS by DVI with the medications regimens to match the vaccinated arms. *Arm 4a* (n=54) will receive 75 mg PYR on days 0 post DVI, *Arm 4b* (n=36) will receive 75 mg PYR on days 2&3 post DVI and *Arm 4c* (n=90) will receive weekly chloroquine prophylaxis +/- NSAIDs.
Participants in the main study will receive three exposures to PfSPZ Challenge (*Arm 1b/5b, 2b/6b* and *3b*) or NS (*Arm 4*), separated by 4 weeks, with PYR or chloroquine coverage (PfSPZ-CVac). The goal in the main study is to assess the safety and tolerability of PfSPZ-CVac (pyrimethamine) regimen in which PYR is dosed earlier with a higher dose of PfSPZ (*Arm 1b/5b*) than previously studied. In addition we will test a PfSPZ-CVac (pyrimethamine) standard regimen with a higher dose of PfSPZ (*Arm 2b/6b*) . In addition, PfSPZ-CVac (chloroquine) regimen at higher dose of PfSPZ will be assessed for safety and tolerability (*Arm 3b*). Participants will also be enrolled in *Arm 4*, a placebo control arm to receive NS injections with either PYR or chloroquine chemoprophylaxis.

Vaccinated participants and non-immunized controls in the main study together will then be monitored for development of parasitemia through the transmission season. All participants will undergo parasitemia clearance with artemether/lumefantrine treatment twice during the course of the study, approximately 2 weeks prior to first and third vaccinations.

In addition, the main study arms will receive a 4^{th} vaccination (booster dose) of $4x10^5$ PfSPZ Challenge or NS to assess safety, tolerability, immunogenicity and protective efficacy of PfSPZ-CVac during a subsequent transmission season. *Arms 1b, 2b,* and *3b* will each receive a single dose of $4x10^5$ PfSPZ Challenge whereas *Arms 4a, 4b,* and *4c* will receive NS while under their respective chemoprophylaxis regimens (PYR on day 0 for *Arms 1b/4a*, PYR on days 2 and 3 for *Arms 2b/4b*, and CQ on days -2 and 5 for *Arms 3b/4c*).

Study Agent/ Intervention Description:

Pyrimethamine: ***Arms 1, 2, 4a, 4b, 5*** **and** ***6*** will receive orally three single strength pills (for a total of 75 mg PYR) per day. The regimen of PYR will be determined in the pilot study dosed on day 0 administered with either $4x10^5$ PfSPZ (*Arm 1a*) or $3x10^5$ PfSPZ (*Arm 5a*) OR on days 2 & 3 with $4x10^5$ PfSPZ (*Arm 2a*) or $3x10^5$ PfSPZ (*Arm 6a*). The placebo control arms will receive NS injections with PYR regimen to match the vaccine groups, 75 mg on day 0 (*Arm 4a*) or on days 2 & 3 (*Arm 4b*). *Arms 3* (chloroquine arm) and *4c* (chloroquine controls) will not receive PYR.

Chloroquine: ***Arm 3 (3a, 3b)*** and ***Arm 4c*** will receive a loading dose of approximately 1000 mg chloroquine (600 mg base) 2 days prior to PfSPZ Challenge or NS injection respectively. After loading dose, *Arm 3a* will receive one additional dose of 500 mg (300 mg base) administered 5 days post PfSPZ Challenge injection, for a total of 2 doses. *Arm 3b* and *4c* will receive 500 mg (300 mg base) weekly continuously with the last dose administered 5 days post 3rd injection. A total of 10 doses of chloroquine will be given to *Arm 3b* and *4c*. *Arms 1, 2*, *4a, 4b, 5* and *6* will not receive chloroquine.

PfSPZ Challenge during PfSPZ-CVac immunization:
In the pilot phase: only 1 dose of $4x10^5$ (***Arms 1a and 2a***) OR $3x10^5$ (***Arms 5a and 6a***) of aseptic, purified, vialed, cryopreserved, fully infectious PfSPZ of PfSPZ Challenge will be administered by direct venous inoculation (DVI). The first day of administration of PfSPZ Challenge is Study Day 1 for each Arm.
In the main phase: 3 doses of $4x10^5$ **(*Arms 1b, 2b and 3b*)** OR of $3x10^5$ (***Arms 5b and 6b)*** of aseptic, purified, vialed, cryopreserved, fully infectious PfSPZ of PfSPZ Challenge will be administered by DVI four weeks apart in the main phase, with a fourth dose given during the booster phase. The first day of administration of PfSPZ Challenge is Study Day 1 thus PfSPZ Challenge will be administered on Study Days 1, 29, 57 and 336.

Normal saline injections:
In the placebo control arms during the main phase (*Arm 4*): 3 doses of NS will be administered by DVI four weeks apart. The first day of administration of NS is

Study Day 1 thus NS will be administered on Study Days 1, 29, 57 and 336.

Primary Objectives:

Safety

Pilot Phase

- To evaluate whether PYR on day 0 prevents patent parasitemia post DVI administration of $4x10^5$ PfSPZ or $3x10^5$ PfSPZ (if necessary) of PfSPZ Challenge (*Arms 1a, 2a, 5a, 6a).*
- To monitor the safety and tolerability of PfSPZ-CVac (chloroquine), post $4x10^5$ PfSPZ of PfSPZ Challenge administered via DVI with chloroquine as the partner drug (*Arm 3a*)

Main Phase and Booster Phase

- To monitor the safety and tolerability of PfSPZ-CVac (PYR), PfSPZ Challenge administered via DVI with PYR as the partner drug; (*Arms 1b/5b, 2b/6b, 4a, 4b)*
- To monitor the safety and tolerability of PfSPZ-CVac (chloroquine), PfSPZ Challenge administered via DVI with chloroquine as the partner drug; (*Arms 3b, 4c*)

Secondary Objectives:

Protective Efficacy

- To assess the protective efficacy of PfSPZ-CVac (pyrimethamine) or PfSPZ-CVac (chloroquine) against natural *P. falciparum* infection (*Arm 1b/5b, 2b/6b, 3b, 4*).

Exploratory Objectives:

Pyrimethamine Regimen Efficacy and Immunogenicity

- To evaluate whether PYR on day 0 or on day 2&3 prevents patent and subpatent parasitemia post DVI administration of $4x10^5$ OR $3x10^5$ PfSPZ of PfSPZ Challenge (*Arms 1, 2, 5, 6).*
- To assess the humoral and cell mediated immune responses to PfSPZ and to known pre-erythrocytic and blood stage antigens *(All Arms)*
- To assess the humoral and cell mediated immune responses to novel pre-erythrocytic antigens *(All Arms)*

- To look for immune correlates of protection
- To assess the kinetics of subpatent parasitemia during PfSPZ-CVac immunization *(Arms 1, 2, 3, 5, 6)*
- To describe changes in γδ T cells in malaria experienced individuals after PfSPZ-CVac immunization and malaria infection during transmission season *(Arms 1b/5b, 2b/6b, 3b)*

Primary Endpoints: **Safety**

Pilot Phase

- Incidence of positive sensitive blood smear (sBS) occurring after PfSPZ-CVac immunization starting on day 7 post DVI (*Arms 1a, 2a, 5a, 6a*).
- Incidence and severity of local and systemic grade 3 signs or symptoms lasting more than 48 hours despite adequate management and serious adverse events (SAEs) occurring after PfSPZ-CVac DVI (*Arm 3a)*

Main Phase and Booster Phase

- Incidence and severity of local and systemic adverse events (AEs) and serious adverse events (SAEs) occurring after PfSPZ-CVac immunization. (*Arms 1b/5b, 2b/6b, 4a, 4b)*

- Incidence of clinical malaria diagnosis occurring after PfSPZ-CVac immunization as defined by the occurrence of grade 3 signs or symptoms lasting more than 48 hours despite adequate management. *(Arm 3b, 4c)*

Secondary Endpoints: **Protective Efficacy**

- *P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood starting 2 weeks after the 3rd vaccination for approximately 6 months (*Arm 1b/5b, 2b/6b, 3b, 4*) in the Main Phase and then 2 weeks after the 4th vaccination for approximately 6 months for the Booster Phase.

Exploratory **Pyrimethamine regimen efficacy and Immunogenicity**

Endpoints:

- *P. falciparum* blood stage infection defined as detection of *P. falciparum* parasites by quantitative polymerase chain reaction (qPCR) following PfSPZ Challenge. (*Arms 1, 2, 5, 6).*
- Humoral immune responses after PfSPZ-CVac regimens by assessing antibodies to PfSPZ, Pf asexual erythrocytic stages (AES), and specific Pf sporozoite, liver and blood-stage antigens such as CSP, merozoite surface protein 1 (MSP-1), AMA-1 in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups. *(All Arms)*
- Cellular immune responses after PfSPZ-CVac regimens to PfSPZ, *Plasmodium falciparum* asexual erythrocytic stages (PfAES), and specific Pf sporozoite, liver and blood-stage antigens, such as CSP, MSP-1, AMA-1, in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups. *(All Arms)*
- Cellular and humoral responses that correlate with protective efficacy
- Prolonged prepatent period and/or reduced density and duration of parasitemia in those developing parasitemia during PfSPZ-CVac immunization *(Arms 1, 2, 3, 5, 6)*
- Comparison of γδ T cells before and after PfSPZ-CVac immunization and and malaria infection during transmission season using *ex vivo* whole blood or *in vitro* staining. *(All Arms)*

Précis

Human studies have shown that immunization by the bite of *Plasmodium falciparum* (Pf) sporozoite (SPZ)-infected mosquitoes (chemoprophylaxis with sporozoites [CPS]) or by injecting aseptic, purified, cryopreserved sporozoites (SPZ) with needle and syringe (PfSPZ chemoprophylaxis vaccination, Sanaria® PfSPZ-CVac) under drug coverage with chloroquine can provide >80% protection against homologous controlled human malaria infection (CHMI) (Roestenberg, McCall et al. 2009, Roestenberg, Teirlinck et al. 2011, Mordmuller, Surat et al. 2017). The protective immunity induced by CPS/PfSPZ-CVac is thought to target sporozoite and liver stage antigens, but the transient parasitemia observed under chloroquine prophylaxis may additionally induce immune responses targeting blood stage parasites that contribute to protection. It is important to understand the contribution of each stage in this model as transient parasitemia in the immunization process adds a safety concern and may impair the development of immunity.

We have conducted two phase 1 studies to explore whether significant protective efficacy can be achieved with exclusively pre-erythrocytic (sporozoite/liver stage) exposure in this model using PYR chemoprophylaxis. This drug, unlike chloroquine, kills the parasites in the liver, so there is no period of transient parasitemia following immunization. These studies in malaria naïve adults demonstrated that PfSPZ-CVac (with PYR administered 2 & 3 days after injection) is safe, well-tolerated, and can prevent subpatent and patent parasitemia after PfSPZ injection. The NCT02511054 study used the same dose ($5.12x10^4$ PfSPZ) that has resulted in >80% protection in previous trials of PfSPZ-CVac (chloroquine), showed only 2/9 vaccinees were protected against a homologous (same strain of Pf as the vaccine) CHMI. In trials using irradiated SPZ, increasing the dose of PfSPZ used for immunization has resulted in higher protective efficacy. Therefore, in an ongoing trial, NCT03083847, we have increased the PfSPZ dose ~4-fold up to $2.0x10^5$ PfSPZ, with preliminary results show markedly improved protection against both homologous and heterologous CHMI in healthy US adults (7/8 and 4/4 study subjects uninfected, respectively). This is the first demonstration that the PfSPZ-CVac approach can confer sterile protection without blood stage exposure.

Building on these promising results from PfSPZ-CVac (pyrimethamine) in the US, this proposed study in a malaria-endemic, adult population in Bancoumana, Mali will initially assess, in a pilot phase, the safety and tolerability of still higher doses of PfSPZ Challenge ($4.0x10^5$ PfSPZ) in combination with PYR (day 0;) or chloroquine (days -2, +5). The higher PfSPZ dose is hypothesized to be necessary to achieve significant efficacy against naturally transmitted malaria in malaria-experienced populations. Once this higher dose of PfSPZ is determined to be safe and tolerable and that earlier PYR dosing prevents patent parasitemia, the main phase will explore the effect of both earlier PYR dosing and a higher dosage of PfSPZ used for immunization in the development of protective immunity against natural malaria infection. The results of this study will contribute to understanding the targets and mechanisms of immunity against Pf malaria infection, and how the degree of prior exposure to the parasite (pre-erythrocytic and/or erythrocytic stages) impacts these responses and subsequent protective efficacy in field settings.

After completion of follow up in the main phase, all participants that are still enrolled in the study will be offered a booster dose of the vaccine (4th dose) with a single dose of $4x10^5$ PfSPZ Challenge or normal saline (aligned with what they received in the main phase of the study) at approximately 11 months post 3rd vaccination. The booster dose is timed to allow administration well before the beginning of the ensuing malaria transmission season. Participants will then be followed for approximately 6 months to assess safety and vaccine efficacy through this second transmission season.

1 Introduction and Rationale

1.1 Malaria Epidemiology

According to the World Health Organization (WHO), global control efforts had resulted in a reduction in the number of malaria cases and deaths; however 435,000 people are estimated to have died due to malaria in 2017, 92% of which occurred in the WHO African Region (WHO 2018), a rate similar to what was reported in 2017. This plateau continues to emphasize the need for additional measures to achieve malaria elimination in endemic countries. Malaria-related morbidity and mortality have a major economic impact in endemic regions and present a significant health risk to non-immune travelers to endemic regions and military personnel deployed overseas. To stem the worldwide impact of this devastating disease, a safe and broadly effective malaria vaccine and improved antimalarial therapeutics are urgently required. Investigations into the development of sterilizing immunity against malaria infection (prevention of blood-stage parasitemia and clinical illness) are of great importance to guide malaria vaccine development efforts and enhance the efficacy of current subunit malaria vaccine candidates.

1.2 Malaria Life Cycle and Vaccine Strategies

Malaria is a vector-borne disease caused by infection with the apicomplexan protozoan parasite, *Plasmodium*. Of the five major species commonly known to infect humans the majority of deaths are caused by *P. falciparum* (Pf). The complex parasite life cycle includes developmental stages in obligate mammalian and insect hosts. The sporozoite (SPZ) form of the parasite is transmitted to humans by the bite of the parasite-infected female *Anopheles* mosquito during a blood meal. The SPZ travel in the bloodstream to the liver, invade hepatocytes and undergo intracellular replication for five to seven days. Arrest of parasite development during this clinically silent pre-erythrocytic or liver-stage would prevent maturation and release of parasites into the bloodstream and limit clinical impact of the disease. Failing this, infected hepatocytes rupture, releasing tens of thousands of merozoite forms into the blood, initiating the pathogenic cycle of erythrocyte invasion and replication (Yoeli 1965). This continuous cycle during the erythrocytic (or blood) stages is responsible for the clinical symptoms of malaria including fever, chills, malaise, myalgia, arthralgia, nausea and vomiting that may progress to severe illness including cerebral malaria, pulmonary edema, renal failure, shock and death.

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 the parasites to mosquitoes. This is the target of leading vaccine strategies including the partially effective recombinant circumsporozoite protein (CSP) based RTS,S vaccine (Mosquirix®), for which phase 3 trials were completed throughout Africa in 2015, and phase 4 and implementation studies are scheduled to start in 2019. Subunit vaccines of this type utilize conserved antigenic targets to elicit protection against SPZ migration, hepatocyte infection and intrahepatocytic parasite replication. RTS,S has protected malaria-naïve adults against experimental Pf controlled human malaria infection (CHMI) and reduced malaria-associated clinical episodes in children living in malaria endemic areas; however, the level and duration of immunity seen is relatively modest (Stoute, Kester et al. 1998, Agnandji, Lell et al. 2011, RTS 2015). In the one study

where the incidence of parasitemia (as opposed to the incidence of clinical episodes) was studied in adults, a study performed in Kisumu, Kenya, RTS,S/AS01b did not induce statistically significant protection (Polhemus, Remich et al. 2009). Although follow up studies of RTS,S vaccine have shown decreases in clinical malaria in vaccinated children, at 6 months post vaccination follow up, the levels of antibodies between vaccinated and unvaccinated children were not significantly different and did not predict protection against clinical malaria in the following 12 months (Campo, Sacarlal et al. 2014). The mechanism by which RTS,S and other SPZ and pre-erythrocytic vaccine strategies confer protective immunity against clinical episodes of malaria is still under investigation, but it is thought that RTS,S primarily induces protective antibody responses targeting SPZ that are dependent on a strong CD4+ helper T cell response, while whole SPZ-based vaccines induce protective CD8+ T cells in the liver that can kill developing liver stage parasites.

In the absence of more effective subunit vaccines, malaria vaccine development has focused on whole organism vaccination to induce sterile immunity. Studies using mosquito bite or needle and syringe to administer radiation-attenuated SPZ (including PfSPZ Vaccine) for vaccination have induced high level (>90%) sterile immunity to Pf in humans (Hoffman, Goh et al. 2002, Roestenberg, McCall et al. 2009, Roestenberg, Teirlinck et al. 2011, Seder, Chang et al. 2013). This required a relatively large number of SPZ to induce protective immunity, e.g. 1000 infectious mosquito bites or five injections of $1.35x10^5$ PfSPZ. In 2009, a human study suggested that anti-infection immunity can be achieved with a much smaller parasite inoculum if *non-attenuated* parasites are used as the immunogen. Roestenberg, et al. demonstrated that wild-type PfSPZ administered 3 times by 12-15 infectious mosquito bites (a total of 36-45 infected mosquitoes) to human subjects receiving concurrent antimalarial prophylaxis with the blood stage drug chloroquine can induce sterile protection against malaria infection upon subsequent challenge with homologous parasites (Roestenberg, McCall et al. 2009). More recently, the same has been shown administering PfSPZ by needle and syringe: three doses of $5.12x10^4$ PfSPZ of PfSPZ Challenge (non-attenuated PfSPZ) in the presence of chloroquine induced 100% protection (9/9 volunteers protected) against CHMI conducted 9-10 weeks after immunization (Mordmuller, Surat et al. 2017), a dose that is 8-fold less than the dose of radiation-attenuated PfSPZ needed to induce high level protection (Epstein, Paolino et al. 2017). The greater potency presumably reflects the fact that non-attenuated SPZ replicate in the liver, greatly increasing the quantity and duration of antigen expression, and concurrently expressing new antigens (mid and late liver stage antigens) that are not expressed by radiation-attenuated SPZ, which halt development early in the liver stages.

This method of immunization by experimental Pf infection with non-attenuated PfSPZ in conjunction with antimalarial prophylaxis is referred to as chemoprophylaxis with SPZ (CPS), infection treatment vaccination (ITV), or chemoprophylaxis vaccination (PfSPZ-CVac) by different authors. We use the term PfSPZ-CVac in this protocol to refer to this vaccination concept that combines the injection by direct venous inoculation (DVI) of aseptic, purified, cryopreserved PfSPZ (PfSPZ Challenge) with oral administration of an antimalarial drug (chloroquine, PYR or others).

1.3 Rationale for Chemoprophylaxis Vaccination (CVac) using Pyrimethamine (PYR) as the Partner Drug

Chloroquine is a selective blood-stage schizonticide and does not kill SPZ or liver stages. Prophylactic doses are sufficient to eliminate all new infections with sensitive malaria strains as they emerge into the blood (Yayon, Vande Waa et al. 1983). Thus the non-attenuated sporozoites (SPZ) used for the PfSPZ-CVac approach when chloroquine is the partner drug develop fully in the liver and are killed only after they emerge into the blood. For this reason, the degree to which the protective immune response induced by PfSPZ-CVac (chloroquine) targets sporozoite, liver, or blood-stage antigens is unclear, and raises the question whether a degree of blood stage immunity is required for the high level protection that is observed. Our hypothesis is that blood stage exposure is not required for protection.

The concept that sterile protective immunity to malaria induced by whole PfSPZ vaccination does not require blood stage immunity is supported by experimental infection studies in both animals and humans. In fact, in rodent malaria models, blood-stage parasites may modulate dendritic cell responses and suppress, rather than bolster, protective CD8+ T cell responses that target parasites infecting hepatocytes (Ocana-Morgner, Mota et al. 2003). The best evidence to date of sterile immunity without blood stage involvement comes from work of Bijker et al at Radboud University Nijmegen Medical Center (RUNMC). Research subjects were immunized by mosquito bite as had been done by Roestenberg et al, and then underwent CHMI by SPZ or by blood stages. There was complete protection against PfSPZ CHMI, but there was no protection against blood stage CHMI, indicating that the immunity induced by CPS was limited to pre-erythrocytic stage antigens (Bijker, Bastiaens et al. 2013). These results are shown in **Table 1**.

Table 1. Protection against blood stage versus sporozoite CHMI after CPS (chloroquine) immunization

				Prepatent period, d, median (range)	
	Challenge	Protected/total no. of volunteers	Protection, %	Thick smear	PCR
Immunized	Sporozoite	5/5	100	N/A	N/A*
	Blood stage	0/9	0	8.0 (7.0–8.3)	5.0 (3.0–5.3)
Control	Sporozoite	0/5	0	12.3 (9.3–12.3)	9.0 (7.0–10.0)
	Blood stage	0/5	0	8.0 (8.0–8.3)	5.0 (2.0–6.3)

*One subject became PCR positive on day 21 after challenge. N/A, not applicable.

^Adapted from Bijker EM, et al. 2013

A second approach to elucidating the stage-specificity of the protective immunity induced by the PfSPZ-CVac approach is to use a partner drug other than chloroquine to kill the parasites in the liver, arresting development before blood stage parasitemia occurs. This should eliminate the possibility that asexual blood stages contribute to the induction of sterile immunity. Several antimalarial drugs with activity against pre-erythrocytic stage parasites that have been considered for PfSPZ-CVac regimens include primaquine, proguanil, and azithromycin. In healthy US adults, we found that a single 45 mg dose of primaquine combined with weekly chloroquine is insufficient to prevent blood stage parasitemia after immunization with PfSPZ Challenge (Healy et al. submitted). Azithromycin has also been

assessed as the PfSPZ-CVac partner drug, and it too was unable to prevent blood stage parasitemia and was not protective upon PfSPZ CHMI (TÜCHMI-002 trial, Mordmuller, Surat et al. 2017). Atovaquone/proguanil has also been assessed, and while this did successfully prevent blood stage parasitemia during immunization, the protective immunity induced was only 20-25%, possibly because the parasites were killed too early in liver stage development (MALACHITE trial, Mordmüller et al, unpublished); therefore the atovaquone/proguanil study was unable to convincingly demonstrate that exclusive exposure to pre-erythrocytic stages of the parasite could induce strong, durable immunity.

Pyrimethamine is an antimalarial drug with both liver and blood stage activity. *Plasmodium berghei* SPZ-CVac (pyrimethamine) regimens in mice have been shown to induce sterile protective immunity (Friesen, Borrmann et al. 2011). Our group at Laboratory of Malaria Immunology and Vaccinology (LMIV) was interested to investigate whether sterile protective immunity could likewise be induced in humans by wild-type (non-attenuated) PfSPZ immunizations when exposure was limited to pre-erythrocytic stages of the parasite life cycle, and selected PYR as the PfSPZ-CVac partner drug for these investigations. We also hypothesized that, as is the case with PfSPZ-CVac (chloroquine), the total immunizing dose of PfSPZ needed with PfSPZ-CVac (pyrimethamine) would be much lower than required for the radiation-attenuated PfSPZ of PfSPZ Vaccine.

We performed an initial human study (#15-I-0169; NCT02511054) with PYR to explore this question of stage-specific immunity. The first finding was that a PfSPZ-CVac (pyrimethamine) regimen using 3 doses of $5.12x10^4$ PfSPZ of PfSPZ Challenge with PYR 50 mg given on days 2 and 3 after each administration of PfSPZ Challenge can prevent subpatent parasitemia completely, as confirmed by negative quantitative reverse transcriptase polymerase chain reaction for Pf parasites. Weekly chloroquine was also administered in this study group as a safety back-up in case parasitemia developed. However, as the results showed that PYR appeared to kill all parasites during liver stage development, it was decided that in future studies PYR could be tested alone. A second finding, from a comparator chloroquine-alone arm, was that participants exposed to $5.12x10^4$ PfSPZ experienced, as expected, detectable subpatent parasitemia after the first PfSPZ Challenge inoculation, with fewer participants being parasitemic after immunizations #2 and #3 than after immunization #1, presumably reflecting the development of immunity. A third finding came when the research subjects underwent CHMI: PfSPZ-CVac (pyrimethamine) did lead to protective immunity, but only 2/9 subjects in the pyrimethamine + chloroquine arm remained uninfected while one additional participant was significantly delayed after homologous CHMI. These two protected volunteers, like the volunteers in the MALACHITE trial, supported the concept that exclusive exposure to pre-erythrocytic stage antigens was sufficient to induce sterile immunity, but the expected robust protection was still not achieved. A fourth finding was to confirm the high level efficacy demonstrated in the prior PfSPZ-CVac (chloroquine) study of Mordmüller et al (Mordmuller, Surat et al. 2017), as 4/5 (80%) subjects in the chloroquine only arm remained uninfected following homologous CHMI. The results of this first PYR study are presented in **Table 2.**

Table 2. PfSPZ-CVac (pyrimethamine) may lead to protective immunity following homologous CHMI

Arm	Number of subjects undergoing CHMI	Number of subjects infected	Days post CHMI to diagnosis	Median days post CHMI to diagnosis	Positive Blood Smear	Percent protected	P-value
1a Pilot (PYR +CQ)	2	2	12, 14	13	1	0%	--
2 (PYR +CQ)	9	7*	8, 11, 12, 12, 12, 14, 18	12	3	11%	>0.7
3 (CQ)	5	1	13	13	0	80%	0.048
4	5	5	8, 9, 12, 12, 12	12	2	0%	--

*One subject was withdrawn from study at Day 16 post CHMI. Up to day 16 post CHMI, the subject remained negative by both blood smear and PCR, thus never documented infected, but since that subject did not complete study follow up per protocol, that subject was considered to have a missing outcome for the binary analysis, but contributed to the time-to-event analysis being censored at day 16.

To improve on these PfSPZ-CVac (pyrimethamine) results, and to build on the concept that larger doses of PfSPZ lead to increased protection, as seen with PfSPZ Vaccine (radiation attenuated PfSPZ), a follow-up study in healthy malaria-naïve US adults was initiated (and remains ongoing) using larger doses of PfSPZ. This ongoing study, (National Institute of Allergy and Infectious Diseases [NIAID] protocol #17-I-0067, NCT03083847) increased the dose of PfSPZ 4-fold from $5.12x10^4$ to $2.0x10^5$ PfSPZ while under PYR alone or chloroquine alone coverage. Malaria-naïve participants were divided into three arms, Arm 2a [PfSPZ-CVac (pyrimethamine) tested by homologous NF54 CHMI n=17]; Arm 2b [PfSPZ-CVac (pyrimethamine) tested by heterologous 7G8 CHMI n=10] and Arm 3 [PfSPZ-CVac (chloroquine) tested by heterologous 7G8 CHMI n=10]. Participants received 3 monthly immunizations with PfSPZ-CVac ($2.0x10^5$ PfSPZ of PfSPZ Challenge) by DVI plus 50mg PYR on 2 and 3 days post injection (Arm 2) or weekly chloroquine until 5 days after the 3rd vaccination (Arm 3)). Homologous CHMI with 3,200 PfSPZ of PfSPZ Challenge (Arm 2a) or heterologous CHMI with 3,200 PfSPZ of PfSPZ Challenge (7G8) (Arm 2b and 3) via DVI was performed approximately 12 weeks post the 3rd PfSPZ-CVac immunization. The main group has been divided into two cohorts and only half of the projected participants have completed the study at this time. The study is expected to be completed in early 2019.

The first completed cohort has now shown promising preliminary results over the first PfSPZ-CVac (pyrimethamine) trial described above. Despite the higher dose of PfSPZ, 50 mg of PYR given on days 2 and 3 after PfSPZ injections was safe, well tolerated, and completely prevented blood stage parasite exposure as evidenced by negative quantitative polymerase chain reaction (qPCR) assays during follow-up period. PfSPZ-CVac (chloroquine) was also safe and tolerable at $1.0x10^5$ PfSPZ, with parasitemia characteristics as expected; however, the higher dose of $2.0x10^5$ PfSPZ resulted in an increase in the density of transient blood stage parasitemia and there were associated Grade 3 adverse events (AEs) on days 7-8 after injection of PfSPZ Challenge. The tolerability of the PfSPZ-CVac

(chloroquine) regimen at the $2.0x10^5$ PfSPZ dose was subsequently much improved with empiric use of non-steroidal anti-inflammatory drugs (NSAIDs) administered presumptively on days 7-8 after PfSPZ Challenge, timed to coincide with the release of asexual blood stages from the liver.

In the first cohort, upon undergoing CHMI with homologous PfSPZ Challenge parasites, 7/8 study subjects immunized with PfSPZ-CVac (pyrimethamine) remained uninfected (see **Table 3** below), a significant improvement from the prior study where smaller immunizing doses of PfSPZ had been used and only 2/9 subjects were protected. In addition, high level *heterologous* immunity was seen for the first time with a PfSPZ-CVac regimen: 4/4 participants in PfSPZ-CVac (pyrimethamine) arm and 3/3 participants in PfSPZ-CVac (chloroquine) arm remained uninfected after heterologous CHMI performed approximately 12 weeks post vaccination #3. The heterologous parasite used was the Plasmodium falciparum, 7G8 clone (Pf7G8), a cloned line from a parasite originally isolated in Brazil (Burkot, Williams et al. 1984). These results, though derived from a small sample size and needing confirmation, demonstrate for the first time that a PfSPZ-CVac regimen involving only pre-erythrocytic exposure (pyrimethamine arms) can induce high grade sterile immunity against both homologous and heterologous parasites for at least three months after completion of vaccination. The finding of heterologous protection is consistent with the performance of PfSPZ Vaccine in the field.

Table 3. PfSPZ-CVac (pyrimethamine) with $2x10^5$ PfSPZ may lead to protective immunity following both homologous and heterologous CHMI

Arm	Subjects undergoing CHMI	Subjects Infected	Days post CHMI to PCR diagnosis	Median days post CHMI to diagnosis	Positive Blood Smear	Percent protected
CVac-PYR (NF54)	8	1	12	12	0	87.5%
CVac-PYR (7G8)	4	0	n/a	n/a	0	100%
CVac-CQ (7G8)	3	0	n/a	n/a	0	100%
NF54 Control	4	4	9, 11, 11, 11	11	1	0%
7G8 Control	4	4	9, 9, 12, 12	10.5	0	0%

Table 3: In order to evaluate whether increasing the dose of PfSPZ in PfSPZ-CVac (pyrimethamine) will result in a higher rate of protective efficacy, we designed a follow-up study, NIAID protocol 17-I-0067, in which malaria-naïve participants were vaccinated with $2x10^5$ PfSPZ, a 4-fold increase over the previous study. In this ongoing study, the main group is divided into three arms, *Arm 2a* [PfSPZ-CVac (pyrimethamine) tested by homologous NF54 CHMI n=17]; *Arm 2b* [PfSPZ-CVac (pyrimethamine) tested by heterologous 7G8 CHMI n=10] and *Arm 3* [PfSPZ-CVac (chloroquine) tested by heterologous 7G8 CHMI n=10]. Participants received 3 monthly immunizations with PfSPZ-CVac ($2x10^5$ PfSPZ of PfSPZ Challenge) by DVI plus 50mg pyrimethamine on 2 and 3 days post injection (*Arm 2*) **or** weekly chloroquine until 5 days after the 3rd vaccination (*Arm 3*)). Homologous CHMI with 3,200 PfSPZ of PfSPZ Challenge (*Arm 2a*) or heterologous CHMI with 3,200 PfSPZ of PfSPZ Challenge (7G8) (*Arm 2b*

and *3*) via DVI was performed approximately 12 weeks post the 3rd PfSPZ-CVac immunization. The main group has been divided into two cohorts and only half of the projected participants have completed the study at this time. In *Arm 2a*, 7/8 participants remained uninfected post homologous CHMI. In *Arms 2b and 3*, 4/4 and 3/3 participants, respectively, remained uninfected post heterologous CHMI. These preliminary results show for the first time that PfSPZ-CVac (pyrimethamine) or PfSPZ-CVac (chloroquine) can achieve sterile immunity to heterologous CHMI, and can achieve sterile immunity in the absence of blood stage parasite exposure [PfSPZ-CVac (pyrimethamine)]. The study is expected to be completed in early 2019.

The potency of PfSPZ-CVac in malaria-naïve adults, now demonstrated with both chloroquine and PYR as the partner drugs, raises the question whether this approach might also provide strong protective immunity in the field, when tested against naturally acquired malaria infection, particularly if doses were increased further to overcome the effects of naturally acquired immunity. An initial study of PfSPZ-CVac (chloroquine) based on this hypothesis has already been done in a partnership between DMID (NIAID), the University of Maryland, and Malaria Research and Training Center (MRTC) at the University of Bamako in Mali (NCT02996695). Participants received 3 monthly immunizations with PfSPZ-CVac (chloroquine) [$2.048x10^5$ PfSPZ of PfSPZ Challenge] or normal saline (NS) administered by DVI using a double-blind design. The chloroquine was administered weekly to both vaccine and placebo groups, with a last chloroquine dose administered 5 days after the 3rd immunization. Immunization was done during the dry season, and protective immunity was assessed against natural transmission over 24 weeks during the ensuing wet season. Although previous PfSPZ-CVac studies to this point had been conducted with $5.12x10^4$ PfSPZ, it was thought that increasing the dose of PfSPZ to $2.048x10^5$ was necessary in this malaria endemic population in order to overcome pre-existing antimalarial immunity in Malian participants, to convey long-term protection over 24 weeks of follow-up, and to overcome malaria parasite antigenic diversity seen in malaria-endemic areas. During the 6 months of follow up post immunization, fewer vaccinated participants (16 of 29, or 55%) were infected than controls (22 of 30, or 73%), but this difference was not statistically significant in this small study (n=31 per group). Vaccine efficacy (VE) by the proportional method was estimated as 0.248 (95% C.I. [-0.048, 0.543]; p = 0.100), and VE by the hazard ratio method was estimated to be 0.336 (95% C.I. [-0.279, 0.655]; p = 0.221). Although there was no statistically significant difference in the incidence of malaria parasitemia between the vaccinated and the control groups, immunization resulted in lower infection rates, indicating that further development of this approach is merited, especially if still larger doses of PfSPZ might improve outcome.

This proposed study builds on this experience. Use of PYR is attractive because it potentially eliminates the period of parasitemia following immunization that is a safety concern associated with PfSPZ-CVac (chloroquine). Our approach to testing PfSPZ-CVac (pyrimethamine) in endemic areas is to first test a more practical regimen in which PYR is administered on the same day (as opposed to administration on 2 and 3 days post injection that has been used in the malaria naïve population to date) as the injection with $2.0 x10^5$ PfSPZ in a pilot study. We also suspect that a higher dose of PfSPZ Challenge will be needed in both PYR and chloroquine arms in this population, owing to evidence that vaccine responses including PfSPZ responses are often suppressed in malaria-exposed populations, thus we will test that the higher dose is safe and well tolerated in the pilot study before proceeding to the main study. The main study will assess the efficacy of these various regimens against naturally transmitted malaria.

1.4 Previous Human Experience

1.4.1 Whole SPZ Vaccination

Sanaria Inc. has developed methods to produce aseptic, purified, cryopreserved PfSPZ that are manufactured according to current Good Manufacturing Practices (cGMP) and are compliant with FDA and EMEA guidances regarding investigational products for human testing. Several PfSPZ-based products have been made, including non-attenuated PfSPZ [PfSPZ Challenge (NF54) and PfSPZ Challenge (7G8), used for CHMI and for PfSPZ-CVac immunization], PfSPZ Vaccine (radiation-attenuated SPZ used to immunize), PfSPZ-GA1 (a genetically attenuated parasite used to immunize) and PfSPZ-GA2 (a second genetically attenuated parasite) (Richie, Billingsley et al. 2015). The manufacturing, formulation, vialing and cryopreservation of each product is identical, except for the irradiation step that is added for PfSPZ Vaccine.

Considering all clinical experience with these products, as of June 2018, 2040 individuals have received 5,403 injections with PfSPZ Challenge, PfSPZ Vaccine or PfSPZ-GA1. Safety and tolerability data indicate that these products are safe and well tolerated. After disappointing results in early trials where PfSPZ Challenge and PfSPZ Vaccine were administered by subcutaneous (sc), intradermal (ID), intramuscular (IM), routes, all PfSPZ-based products are now administered by DVI. The most advanced Sanaria product, PfSPZ Vaccine, has been studied in 18 clinical trails, including 7 double blind, placebo-controlled trials conducted in African populations, and there have been no significant differences between vaccinees and NS placebo controls by any safety measure, including solicited AEs, unsolicited AEs and laboratory abnormalities. The trials conducted in malaria-naïve adults in the USA have not included blinded control groups, but in general, findings are consistent with the African trials: low rates of AEs that likely reflect background rates. In summary, during the course of 16 studies of PfSPZ Vaccine where immunizations have been completed (not counting recently initiated trials in children in Gabon and adults in Mali), 1157 research subjects have safely received 3786 doses by sc, ID, IM, intravenous via catheter (IV) or DVI routes (as high as $2.2x10^6$ PfSPZ im and $2.7x10^6$ PfSPZ by DVI), comprising 652 adults and 505 children. Discounting ID, IM and IV catheter routes, this includes 448 adults age 18-65 years, 12 children age 11-17 years, 12 children age 6-12 years, 36 children age 1-5 years, and 400 infants age 5-11 months, who have received PfSPZ Vaccine by DVI. PfSPZ Vaccine has proven safe and well tolerated, with no AEs or allergic reaction clearly linked to the vaccine. The favorable safety and tolerability profile of PfSPZ Vaccine and other PfSPZ-based products has helped to accelerate their development.

The only AEs that are clearly a result of injection with PfSPZ are the malaria-related symptoms that occur in conjunction with the parasitemia that develops after injection of PfSPZ Challenge. When PfSPZ Challenge is used to immunize as part of the PfSPZ-CVac approach, with chloroquine as the partner drug, these adverse parasitemia-related signs and symptoms occur 7-9 days after injection of PfSPZ Challenge but end quickly as the parasites are killed by the chloroquine, and generally are markedly reduced after the second and third immunizations compared to after the first, because of the rapid development of immunity. These side effects are discussed in more detail below.

1.4.2 PfSPZ Challenge (NF54) for Immunization

An important role of PfSPZ Challenge (NF54) is its use as the immunogen in the PfSPZ-CVac approach to vaccinating against malaria. To date, 8 clinical trials of PfSPZ-CVac using PfSPZ Challenge have been performed (or are underway), varying the route of administration, the dose size, the interval between doses, and the partner drug. These trials are summarized in **Table 4** below:

Table 4. Chronological Listing of Trials of the PfSPZ-CVac Approach to Immunization

Name	Site Institution	First injection	N	Route	Partner drug	# doses	Highest # PfSPZ	Interval betweeen injections	Best protection*
1. TIP-5 NCT01728701	Nijmegen Radboud UMC	Sep 2012	20	id	CQ	3-4	$7.5x10^4$	4 weeks	0% (0/20)
2. TÜCHMI-002 NCT02115516	Tübingen Inst for Trop Med	Apr 2014	45	DVI	CQ, or CQ + azithromycin**	3	$5.12x10^4$	4 weeks, 2 weeks, 5 days	100% (9/9) 67% (6/9) 63% (5/8)
3. 15-I-0169 NCT02511054	Bethesda NIAID / LMIV	Nov 2015	20	DVI	CQ, or CQ + pyrimethamine	3	$5.12x10^4$	4 weeks	80% (4/5) (CQ alone)
4. MALACHITE NCT02858817	Tübingen Inst for Trop Med	Nov 2016	21	DVI	atovaquone / proguanil	3	$1.5x10^5$	4 weeks	27% (3/11)
5. DMID 11-0042 NCT02773979	Seattle Group Health	Jan 2017	21	DVI	CQ	3	$1.024x10^5$	7 days 5 days	0% (0/8) 75% (6/8)
6. 17-I-0067 NCT03083847	Bethesda NIAID / LMIV	Jun 2017	51	DVI	CQ, or pyrimethamine	3	$2.0x10^5$	4 weeks	87.5% (7/8) pyrimethamine homologous 100% (4/4) pyrimethamine heterologous 100% (3/3) CQ heterologous
7. EGSPZV2 NCT02859350	Equatorial Guinea Malaria Vaccine Initiative (EGMVI), Baney Clinic, EG	2017	26	DVI	CQ	3	$1.0x10^5$	4 weeks	Pending (homologous CHMI completed, data cleaning in process).
8. DMID 11-0042 NCT02996695	Bamako Malaria Research Training Center, Mali / LMIV	Part A 2017	36	DVI	CQ	3	$2.048x10^5$	4 weeks	Not applicable. Safety study
		Part B 2017	62	DVI	CQ	3	$2.048x10^5$	4 weeks	37.7% (proportional), 47.1% (time-to-event) over 24 weeks
Total subjects:			302 (includes placebo controls)						
* Percent protected (# protected/# undergoing CHMI). **Protection was in CQ alone in groups.									

1.4.2.1 PfSPZ-CVac under Chloroquine Prophylaxis in Malaria-Naïve Populations

The first trial listed was performed in The Netherlands before it was known that id administration was suboptimal. PfSPZ-CVac (chloroquine) was tested using the id route, and although the PfSPZ were safe and well tolerated, there was no protection (Bastiaens, van Meer et al. 2016).

Subsequently, a randomized, placebo controlled, double-blinded, dose finding trial of PfSPZ-CVac (chloroquine) was performed in Germany using DVI administration (TÜCHMI-002 trial, NCT02115516) to identify the dose needed to replicate the 100% protection results see following mosquito-bite immunization in *Roestenberg et al.* In stage A of the TÜCHMI-002 trial, three groups (n=9 in each) received three PfSPZ Challenge administrations 4 weeks apart by DVI with 3,200, $1.28x10^4$ or $5.12x10^4$ PfSPZ of PfSPZ Challenge while under chloroquine prophylaxis. The latter was administered weekly for a total of 10 doses, starting with a loading dose 2 days before the first injection of PfSPZ Challenge and ending with a last dose given 5 days after the third injection of PfSPZ Challenge. A fourth group (n=9) received placebo injections in this double blind study. Nine to ten weeks after immunization, homologous CHMI was performed with 3,200 PfSPZ of PfSPZ Challenge administered by DVI, and 3/9 (3,200 PfSPZ-CVac group), 6/9 ($1.28x10^4$ PfSPZ-CVac group) and 9/9 ($5.12x10^4$ PfSPZ-CVac group) of the subjects respectively were protected (Mordmuller, Surat et al. 2017). The results of the high dose group reproduced the 100% protection seen in *Roestenberg et al* and highlighted the importance of a sufficient antigen load required for VE. The investigation also demonstrated that a sufficient antigen load to achieve 100% protection is multifold lower when compared with the irradiated PfSPZ of PfSPZ Vaccine (Seder, Chang et al. 2013).

Safety data from this study after unblinding showed that AE rates between the four treatment groups were not significantly different from each other. The events/laboratory abnormalities that were solicited/studied included pain, tenderness, erythema/redness, and induration/swelling at the injection site; fever (°C), tachycardia, bradycardia, hypertension (systolic, diastolic), abnormal respiratory rate; nausea/vomiting, diarrhea, headache, fatigue, myalgia; and abnormal levels of sodium, potassium glucose, bilirubin, creatinine (Cr), LDH, alanine transaminase (ALT), aspartate aminotransferase (AST), white blood count differential component count including neutrophils, lymphocytes, monocytes, basophils, eosinophils, erythrocyte count, hemoglobin, hematocrit. Rates of AEs are shown in **Figure 1**. There were no significant differences in the rates of AEs between the three injections of PfSPZ Challenge (data not shown) in any of the groups.

Figure 1. Frequency of adverse events in all 4 groups after PfSPZ Challenge DVI

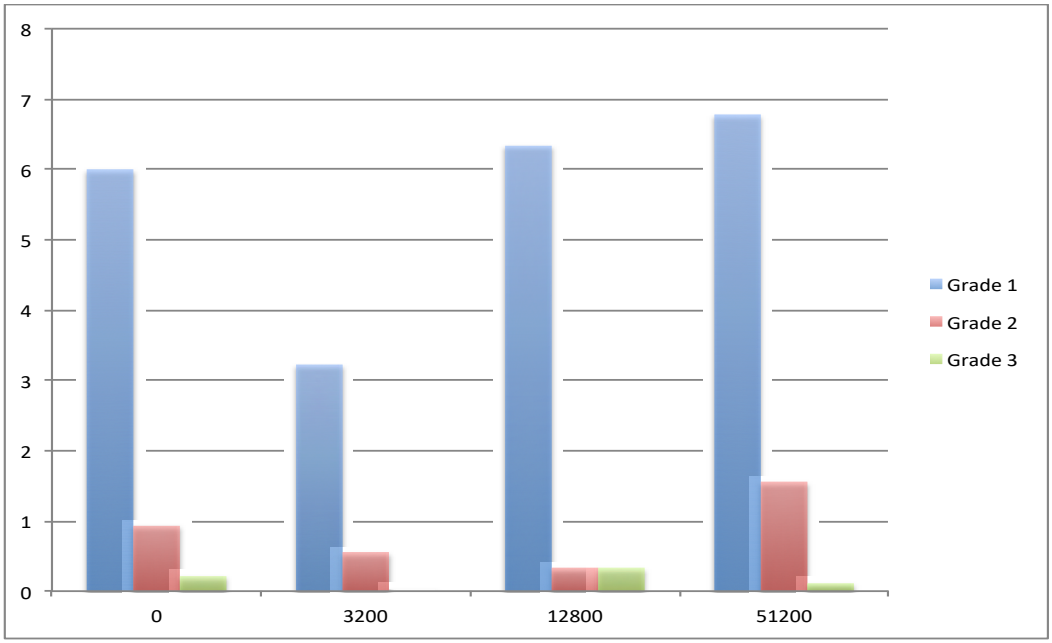


Figure 1. Total number of solicited and unsolicited adverse events (including abnormal signs and laboratory values) recorded per volunteer during administration of 3 doses of vaccine or placebo (y axis), in each PfSPZ dose group (x axis). The period covered is from day -3 to day 77.

Parasitemia was seen by qPCR in all PfSPZ-immunized volunteers after the first immunization with PfSPZ Challenge (except one of the volunteers in the group receiving 3,200 PfSPZ where technical issues prevented completion of the assay for this sample), and in a decreasing number of volunteers after the second and third immunizations, this reduction especially apparent in the highest dose group (**Table 5**). Parasitemia typically occurred during the 6-10 day interval after PfSPZ Challenge administration, peaking on days 7 or 8.

Table 5. Summary of PfSPZ dose sizes during PfSPZ-CVac (chloroquine) immunization and the number of qPCR positive subjects following each immunization

PfSPZ Challenge dose per immunization (3 PfSPZ)	**Number Subjects Positive by qPCR days 6-10**		
	Immunization 1	Immunization 2	Immunization 3
3,200	8 / 8*	9 / 9	6 / 9
$1.28x10^4$	9 / 9	8 / 9	7 / 9
$5.12x10^4$	9/ 9	8 / 9	3 / 9

* Technical difficulties precluded assessment of one volunteer's sample

An important, even if expected, finding of the study was that the density of parasites during the period of transient parasitemia increased with increasing doses of PfSPZ Challenge, approximately doubling with each 4-fold increase in PfSPZ dose. The geometric mean parasite densities (ranges) after the first immunizations in the low, medium and high dose groups in TÜCHMI-002 were, respectively, 462 (228-989), 711 (120-1,891) and 15,652 (5,146-36,404) parasites/mL (**Table 6**).

Table 6

Table 6. Summary of PfSPZ dose sizes and mean parasite density after each immunization

	Geometric Mean Parasite Density (range)*		
Dose (PfSPZ)	Immunization 1	Immunization 2	Immunization 3
3,200	462 (228-989)	78 (2-1,236)	210 (31-2,170)
$1.28x10^4$	711 (120-1,891)	195 (13-762)	57 (14-417)
$5.12x10^4$	15,652 (5,146-36,404)	555 (63-3,089)	42 (11-126)

* parasite equivalents per mL, including only those subjects who were positive by PCR

The highest parasitemia identified in the TÜCHMI-002 trial followed the first immunization in a volunteer in the high dose group: 36,404 parasites/mL. Densities above 10,000 per mL may be positive by thick blood smear, and may lead to malaria symptoms. Nevertheless, thick blood smear readings were negative in this volunteer and in all volunteers at all time points. The reason for the negative blood smear in the volunteer with 36,404 parasites/mL (which we expected should have been positive) may have related to the moribund state of the parasites (DNA positive by PCR, but morphology distorted on thick smear).

The volunteer experiencing the density of 36,404 had an axillary temperature to 38°C and symptoms of fever, fatigue and tachycardia on days 8–10 in association with this parasitemia. While this volunteer later developed a urinary tract infection, the investigators considered that the fever and symptoms were nevertheless potentially related to the parasitemia. With the exception of this volunteer, there were no increases above background in the frequency of malaria-related symptoms such as fever, headache or myalgia during the periods of parasitemia, in any groups.

Thus, in summary, there was no clear indication that immunization with up to $5.12x10^4$ PfSPZ of PfSPZ Challenge caused any excess of abnormal signs, symptoms or laboratory values relative to NS placebo, with the possible exception of one volunteer in the $5.12x10^4$ PfSPZ group.

In stage B of the study, condensed regimens were studied: three doses of $5.12x10^4$ PfSPZ were administered to 19 volunteers divided into three cohorts, and concurrently three doses of NS were administered to 6 volunteers in blinded fashion (two per cohort). The timelines for the regimens were shortened relative to Stage A: 10 volunteers received doses of PfSPZ Challenge on days 0, 14 and 28, and 9 volunteers received doses of PfSPZ Challenge on days 0, 5 and 10. Half of the 10 volunteers on the four-week schedule received 2g extended release azithromycin (Zithromax UNO, Pfizer) in addition to chloroquine with the first dose of PfSPZ Challenge, to see whether azithromycin could prevent the development of parasitemia during immunization. Azithromycin was not successful in preventing parasitemia, so its use was dropped for the second and third immunizations. AE profiles on days 7-10 after each immunization were similar to those seen in Stage A, and did not suggest that parasitemias were high enough to be symptomatic. CHMI was conducted using PfSPZ Challenge administered by DVI 10-11 weeks after the last immunization, and 6/9 (67%) volunteers were protected after the 0, 14 and 28 day regimen, and 5/8 (63%) volunteers were protected after the 0, 5 and 10 day regimen. These results showed that the PfSPZ-CVac approach induced sterile immunity even after highly condensed regimens, although there was a reduction in VE compared to the 100% protection seen with the 0, 28 and 56 day regimen.

There was one unanticipated safety issue. One of the volunteers receiving the 0, 14 and 28 day regimen of PfSPZ Challenge experienced parasitemia by PCR on day 7, peaking on day 8 and then falling progressively on days 9 and 10, which is the typical pattern. But then atypically, the subject showed increasing parasitemia on day 11, and was found to be blood smear positive on day 13, during which time the subject also experienced mild symptoms of malaria (headache, sweating). The volunteer was treated with atovaquone/proguanil on days 13-15 and cleared the parasitemia. A retrospective review of the day 11 blood smear with more intensive searching revealed trace parasites. This subject's plasma chloroquine levels on day 13 were less than 2 ng/mL (certified limit of assay quantification 5 ng/mL) although trace levels of chloroquine were present. On questioning, the volunteer stated that he did swallow the chloroquine tablets. The team proposed a pharmacokinetic study of chloroquine absorption and metabolism in this volunteer to explain the apparent poor bioavailability. The volunteer initially agreed to undergo this study, and to take another dose of 10 mg/kg chloroquine with additional blood samplings to diagnose possible causes of the low plasma levels. However, when the pharmacokinetic study had been set up, the volunteer refused to participate. Therefore, the reason for the low chloroquine levels in this volunteer may never be definitively determined. The suspicion is that the volunteer kept the chloroquine tablets in his cheek and discarded them later. The small amount of absorption that would have taken place through the oral mucosa would explain the trace levels found on day 13. Post-ingestion oral examinations for retained tablets were not performed in this study.

The parasites from this volunteer were isolated on day 13 and tested *in vitro* for chloroquine resistance, and were found to be highly sensitive to chloroquine, consistent with all testing that has been performed on PfSPZ Challenge in the past. As a result of this unanticipated event (inadequate levels of chloroquine), subsequent studies measured levels of chloroquine prior to anticipated peaking of parasitemia. To date, the measured levels have been adequate. Chloroquine levels will thus not be measured in the current study. The safety issue in this volunteer is deemed related to poor compliance, and since this event, oral examinations have been performed to confirm chloroquine ingestion. Rigorous follow-up is always done starting on day 7 after immunization, since any uncontrolled parasitemia should quickly manifest as clinical signs and symptoms, and, because of daily follow-up, does not involve any greater risk than the risks of CHMI.

Although PfSPZ-CVac (chloroquine) with $5.12x10^4$ PfSPZ of PfSPZ Challenge in three successive 4-weekly vaccinations led to 80-100% protective efficacy against homologous CHMI (the result depending on the intervals between injections,) it is likely that a higher dose of NF54 PfSPZ Challenge will be required to achieve protection against heterologous CHMI. As mosquitoes in endemic areas harbor multiple strains of Pf, it will be important to demonstrate that vaccine candidates can protect against heterologous strains.

Therefore, a phase 1, blinded, randomized, dose escalation trial to assess the safety and efficacy of PfSPZ-CVac (chloroquine) using higher doses of PfSPZ was initiatiated, recruiting healthy malaria-naïve adults in Seattle (Clinicaltrials.gov Identifier: NCT02773979). The plan was for 3 study groups to receive doses of $5.12x10^4$, $1.024x10^5$ or $2.048x10^5$ PfSPZ respectively. Each group targetted 12 subjects randomized 3:1 to PfSPZ Challenge or NS, and was to receive 3 vaccinations at 7 day intervals (days 0, 7, 14). This condensed weekly regimen, similar to (but not identical to) the 5 day interval regimen studied in Part B of the

TÛCHMI-002 trial, was selected for the Seattle trial because of the practicality of rapid immunization schedules and the convenience of coming in for an immunization on the same weekday each week for three weeks. Enrollment of successive groups for dose escalation was staggered, with each higher dose group to be enrolled after the lower dose group had received all three vaccinations.

The first study group raised unexpected safety concerns. Subjects in the low dose group ($5.12x10^4$ PfSPZ at 7 day intervals) experienced a higher frequency and severity of AEs (**Table 7)** in comparison to the PfSPZ-CVac (chloroquine) studies where the same dose had been given at 4 week, 14 day or 5 day intervals (NIAID protocol # 15-I-0169; (Mordmuller, Surat et al. 2017). There were 2, 7 and 1 Grade 3 (severe) AEs after vaccinations 1, 2 and 3, respectively, and no Grade 3 AEs had occurred in our study at National Institutes of Health (NIH) or in Germany. These included high grade fever, severe malaise, severe myalgia, severe arthralgia and severe chills, all occurring approximately 8 days after receiving the first, second or third injection with PfSPZ Challenge, and coinciding with periods of transient parasitemia. Due to the AEs, one volunteer was dropped out prior to the third immunization, reducing the sample size from 9 to 8. One potential explanation for the unexpected frequency of Grade 3 AEs was that there was exaggerated cytokine release due to the administration of a large number of PfSPZ in the presence of blood stage parasites, which peaked in density on roughly the seventh and eighth days after each injection of PfSPZ Challenge.

Table 7. List of AEs post PfSPZ-CVac (chloroquine) immunization with weekly $5.12x10^4$ PfSPZ of PfSPZ Challenge administered by DVI in NCT02773979

Study day*	Days post PfSPZ Challenge Injection****						
	Post CVac #1	Post CVac #2	Post CVac #3	Grade 1	Grade 2	Grade 3	Total
3 - 9 (CVac #1, n=9)	0 to 6	N/A	N/A	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
10 - 16 (CVac #2, n=8)	**7 to 13**	0 to 6	N/A	29 (6) 75%	4 (2) 25%	2 (1) 13%	35 (7) 88%
17 - 23 (CVac #3, n=8**)	14 to 20	**7 to 13**	0 to 6	36 (7) 88%	20 (6) 75%	7 (2) 25%	63 (7) 88 %
24 - 25 (post CVac #3, n=6***)	21 to 22	14 to 15	**7 to 8**	16 (4) 67%	4(2) 33%	1 (1) 17%	21 (4) 67 %

Note: X (X) X% refers to Absolute number of AEs (Number of subjects with AEs) Percent of subjects with AEs

* vaccinations were performed on days 3, 10 and 17, so periods of parasitemia initiated 7 days later, i.e., days 10, 17 and 24.

** 1 subject did not receive Vax #3 but was followed for safety

*** Data only available for 6 subjects followed up to study day 25, 22 days post PfSPZ-CVac immunization #1

**** Malaria symptoms are expected around 7-8 days post injection, corresponding to the period of transient parasitemia

Due to these adverse findings, the design of the study was modified and the highest dose administered for vaccination was limited to $1.024x10^5$ PfSPZ. The third group reverted to 5 day intervals between injections, a scenario where PfSPZ Challenge injections #2 and #3 are done prior to, not concurrently with, the period of transient parasitemia resulting from the prior injection, which does not start until the seventh day. Consistent with the parasitemia hypothesis, the AE profile of this group greatly improved, with no Grade 3 AEs recorded. Efficacy results further supported the hypothesis that PfSPZ Challenge should not be injected during parasitemia: the protective efficacy of Group 1 against CHMI at week 10 was 0% (0/7 volunteers protected), whereas for Group 3 ($1.024x10^5$ PfSPZ at 5 day intervals), protective efficacy was 75% (6/8 volunteers protected), similar to the results of the 5-day interval assessed in the TÜCHMI-002 trial (63%, see above). Group 2 received $1.024x10^5$ PfSPZ at 7-day intervals with better tolerability than Group 1, but did not undergo CHMI due to the absence of protection seen in Group 1.

1.4.2.2 PfSPZ-CVac (chloroquine) in Malaria Experienced Populations in Mali and Equatorial Guinea

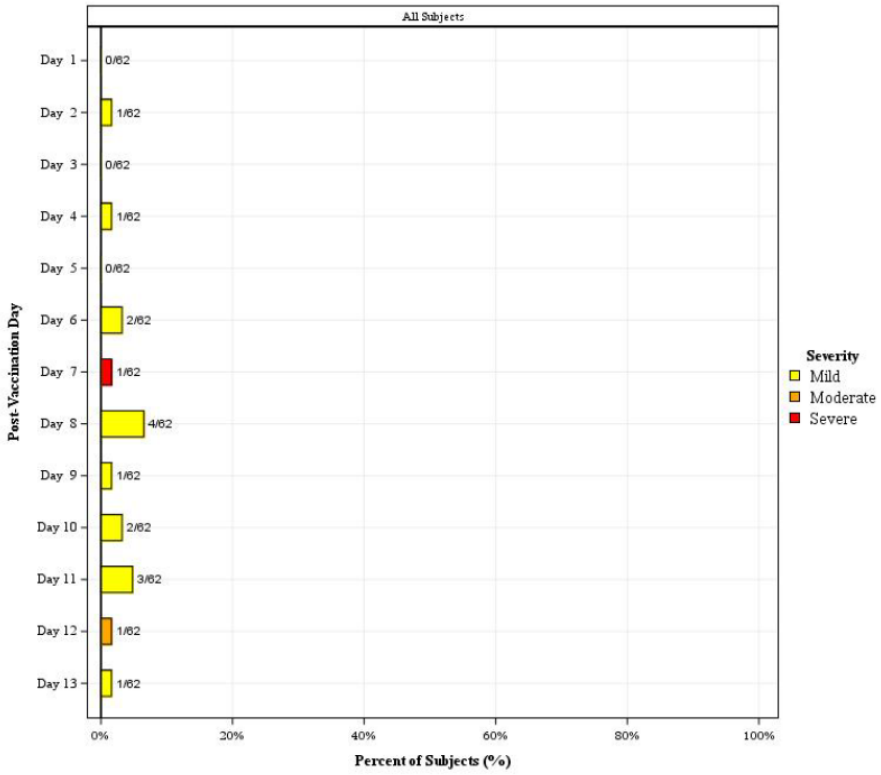
The good tolerability and excellent protective efficacy of PfSPZ-CVac (chloroquine) in malaria naïve populations from the MAVACHE trial in Germany and in Group 3 in Seattle led to a study to assess the development of protective efficacy against natural transmission. This was a randomized, double-blind placebo controlled single center study conducted by MRTC in Mali (Clinicaltrials.gov: NCT02996695) to evaluate the safety, tolerability, immunogenicity and efficacy against naturally occurring malaria in malaria-exposed Malian adults. Participants were randomized into two groups of 31 research subjects to receive 3 monthly injections of either $2.048x10^5$ PfSPZ of PfSPZ Challenge or NS by DVI under weekly chloroquine prophylaxis. The last dose of chloroquine was given at 5 days post vaccination #3 followed by clearance with artesunate monotherapy for 7 days. Follow up to record incident natural malaria infection was started two weeks later. As briefly outlined in **Section 1.3**, during the 6 months of follow up post immunization, fewer vaccinated participants (16 of 29, or 55%) were infected than controls (22 of 30, or 73%), but this difference was not statistically significant.

The regimen was safe and well tolerated. Reviewing the blinded data (31 vaccinees and 31 placebo recipients combined), a total of 32 subjects experienced at least one solicited AE within 12 days following any dose. Of the 32 subjects, 13 experienced at least one solicited systemic AE, and 24 subjects experienced at least one solicited local AE, reported on the day of vaccination. All the local solicited AEs were mild (**Table 8**). All systemic AEs were mild, except in the case of one subject who experienced a Grade 3 (severe) fever on Day 7 after the first immunization. This AE, along with a Grade 2 (moderate) headache occurring in the same individual, were assessed by the investigator as unrelated to immunization and related to an intercurrent upper respiratory infection (**Figure 2**). There was 1 serious adverse event (SAE) that was not related to the study that occurred 157 days after immunization; the participant had a motorcycle accident that resulted in death due to cranial trauma.

Table 8. Number and Percentage of subjects experiencing solicited events with 95% confidence intervals by symptom and treatment group

	All Subjects (N=62)		
Symptom	**n**	**%**	**95% CI**
Any Symptoms	**32**	**51.6**	**(39.45, 63.59)**
Any Systemic Symptoms	**13**	**21.0**	**(12.68, 32.64)**
Arthralgia	1	1.6	(0.29, 8.59)
Chills	1	1.6	(0.29, 8.59)
Fever, Subjective	2	3.2	(0.89, 11.02)
Headache	6	9.7	(4.51, 19.55)
Malaise	5	8.1	(3.49, 17.53)
Myalgia	0	0.0	(0.00, 5.83)
Nausea	1	1.6	(0.29, 8.59)
Temperature Elevation	1	1.6	(0.29, 8.59)
Any Local Symptoms	**24**	**38.7**	**(27.58, 51.15)**
Bruising at Injection Site	0	0.0	(0.00, 5.83)
Erythema/Redness at Injection Site	0	0.0	(0.00, 5.83)
Induration/Swelling at Injection Site	1	1.6	(0.29, 8.59)
Pain at Injection Site	24	38.7	(27.58, 51.15)
Tenderness at Injection Site	0	0.0	(0.00, 5.83)
Note: N=Number of subjects in the Safety Analysis Population			

Figure 2. Maximum severity of solicited systemic symptoms per subject by day post treatment

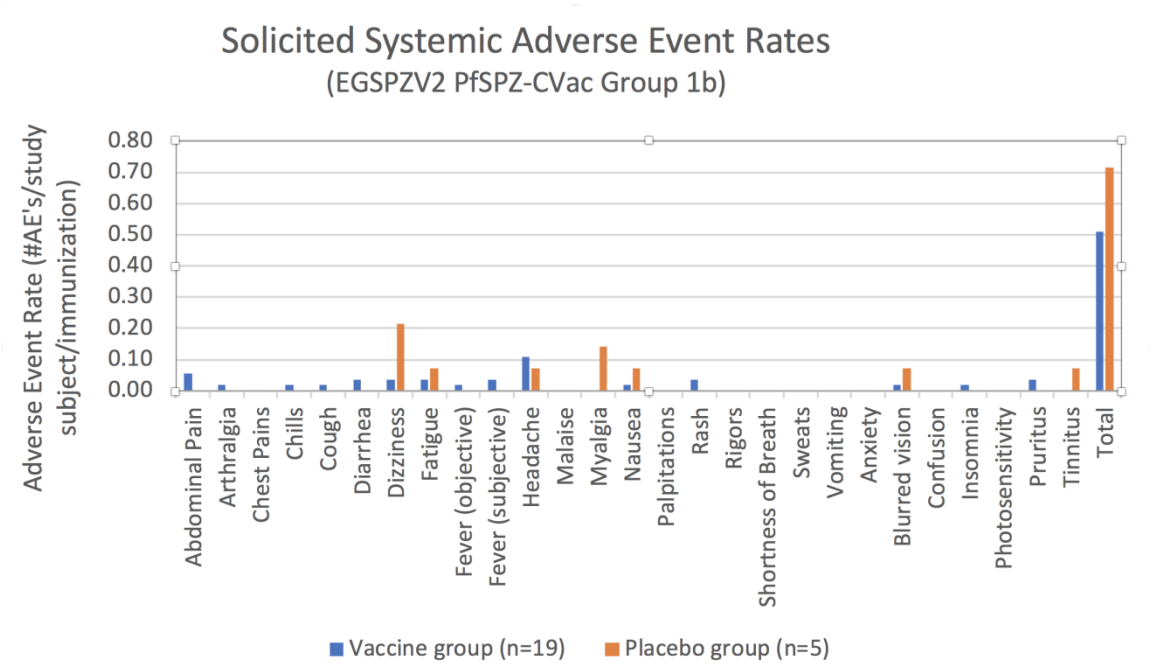


Unblinded safety data from this Mali trial are not available at the current time. However, the frequency of moderate or severe AEs was low. Depending on the treatment assignment of the volunteer experiencing the one Grade 2 and the one Grade 3 systemic AE, the frequency was either 1/31 or 0/31 for Grade 2 or for Grade 3 AEs over the course of three immunizations, with a dose of PfSPZ ($2.048x10^5$) that was twice as high as the highest dose studied in Seattle ($1.024x10^5$ PfSPZ), and four times as high as the highest dose studied in the TÜCHMI-002 trial in Germany and the chloroquine alone group in the first NIH PYR trial ($5.12x10^4$ PfSPZ). Regarding Grade 1 AEs, a worst case scenario (all systemic AEs occurring in the vaccine group and none in the placebo group), the frequency of Grade 1 AEs *occurring on days 7-9* after immunization was 6/31 (19.4%).

These safety data indicate that the semi-immune status of the volunteers in the Mali trial impacted the tolerability of PfSPZ-CVac (chloroquine) immunization. Explanations could be (1) the effective dose of parasites was reduced due to neutralization of SPZ, elimination of liver stages or suppression of blood stages, leading to reduce density of parasites in the blood and therefore better tolerability, and/or (2) naturally acquired immunity allowed better tolerability of whatever density of parasitemia was present. It should be possible to better differentiate the contribution of each of these mechanisms once data on the density of parasitemia occurring after each immunization are available from the trial. In any case, the results of the study indicate that higher doses of PfSPZ could be safety studied in this population of semi-immune, malaria-exposed adults.

A second trial in Equatorial Guinea, EGSPZV2 (ClinicalTrials.gov Identifier: NCT02859350), has assessed the safety of PfSPZ-CVac (chloroquine) in African adults in one group of volunteers (out of 8 total groups in the study). The PfSPZ-CVac (chloroquine) group in this trial, Group 1b, started in January 2017, 3 months prior to the Mali PfSPZ-CVac (chloroquine) trial just discussed (protocol 15-0052). Group 1b participants, young adults age 18-35, were randomly assigned to received 3 injections with $1.0x10^5$ PfSPZ of PfSPZ Challenge (NF54) (n=19 for the first immunization, n=18 for the second and third immunizations) or NS placebo (n=5 after the first two immunization, n=4 after the third immunization) at 4-week intervals, using a double blind design. The PfSPZ dose in vaccinees was ~2-fold higher than the highest dose in TÜCHMI-002 and ~half the dose of the protocol 15-0052 in Mali. All Group 1b volunteers were administered chloroquine using the standard regimen, a loading dose 2 days before the first injection of PfSPZ Challenge followed by weekly maintenance doses. Vaccine recipients experienced local (site of injection) AEs on six occasions after the 55 vaccinations (rate 0.1 AE/study subject/injection). The local AEs consisted of mild pain or mild tenderness. No local AEs were reported by NS recipients after their 14 vaccinations. Vaccine recipients experienced systemic AEs on 28 occasions (0.51 AE/study subject/injection) after the 55 vaccinations, and placebo recipients experienced the same on 10 occasions after their 14 vaccinations (0.71 AE/study subject/injection, a slightly higher rate than for vaccine recipients). The systemic adverse event rates per injection are presented in **Figure 3**. There was no statistically significant difference in rates between vaccine and placebo recipients.

Figure 3. Solicited Systemic Adverse Events per injection during EGSPZV2 trial.



The AEs included two episodes of systemic pruritus in vaccinees, both mild in severity, one occurring day 1 after the first immunization (day 3 after chloroquine load) and one occurring day 2 after the first immunization (day 4 after chloroquine load). Both resolved spontaneously without treatment and neither reoccurred with ongoing chloroquine administration or after the second or third vaccination. Both were deemed likely associated with chloroquine, which is reported to cause pruritus in about 50% of dark skinned persons receiving chloroquine for malaria treatment, noting that the prophylactic doses of chloroquine administered to volunteers in the EGSPZV2 trial were lower than treatment doses usually associated with pruritus. Vaccinees experienced 6 episodes of headache (two moderate in severity), 3 episodes of abdominal pain (1 moderate in severity), 2 episodes of diarrhea (1 moderate in severity), 2 episodes of dizziness, and 1 episode of a variety of other symptoms (see **Figure 3**). Rates in placebo recipients were similar to those in vaccinees or not statistically significantly different. There were no concerning trends in hematological (hemoglobin, white blood cells [WBCs], platelets) or biochemical (Cr, ALT and AST) laboratory markers.

Parasite Densities: Twenty-two volunteers completed all three PfSPZ-CVac or placebo immunizations in Group 1b, 1 completed two immunizations and 1 completed only the first immunization. Dropping out from further immunizations was not due to AEs. PCR data (which remain blinded at this point) from the 22 showed that 12 were PCR positive for parasites during all three periods of potential parasitemia (days 6-10 after each injection of PfSPZ Challenge), 1 was positive during the first and third periods, 1 was positive during the first and second periods, and 3 were positive only during the first period. The remaining 5 out of 22 volunteers were PCR negative during all three periods, and appear likely that 4 of these were the NS controls. Two additional volunteers received one or two immunizations and did not show parasitemia, indicating that three true-immunized volunteer did not show parasitemia after the first immunization. This can be deduced because the group includes 4 controls, and 7 volunteers were PCR negative after the first dose of PfSPZ Challenge. Peak parasitemias occurred on days 7 or 8, after either the first or the third immunization (none peaked after the

second), with densities ranging from 0.2 to 10.3 parasites/µL (geometric mean of all peak parasitemias = 1.35 parasites/µL), which was lower than what was observed in the TÜCHMI-002 trial (geomean 15.7 parasites/µL after the first immunization), noting that the dose of PfSPZ Challenge administered in EGSPZV2 was ~ two times higher ($5.12x10^4$ PfSPZ of PfSPZ Challenge in TÜCHMI-002 and $1.0x10^5$ PfSPZ of PfSPZ Challenge in EGSPZV2). This reduction in the density of transient parasitemia despite a higher PfSPZ dose is likely related to natural acquire immunity in these volunteers, who live in a malaria-endemic area, and have experienced life-long exposure. Not surprisingly, given these low densities, no blood smears were positive. Parasitemia data are provided in **Table 9** and **Figure 4**. It was concluded that lower parasite densities certainly contributed to improved tolerability, but there could additionally be a factor of improved tolerability at any given parasite density, relative to malaria-naïve individuals.

Table 9. Parasitemia Indices (Protocol EGSPZV2)

Group			Dose1			Dose 2			Dose 3		
Group Name	Dose	Interval	# pos / # imm	Median peak parasit	Range peak parasit	# pos / # imm	Median peak parasit	Range peak parasit	# pos / # imm	Median peak parasit	Range peak parasit
2b vaccinees	$1.0x10^5$	4 weeks	17/18	0.758	0-10.284	13/18	0.456	0-2.169	15/18	1.037	0-10.278

Table 9: the number of study subjects with parasitemia detected after each immunization relative to the number injected, the median peak parasite density (including zero values, in parasites/µL), and the range in peak density (including zero values, in parasites/µL).

Figure 4. Parasitemia Curves (Protocol EGSPZV2)

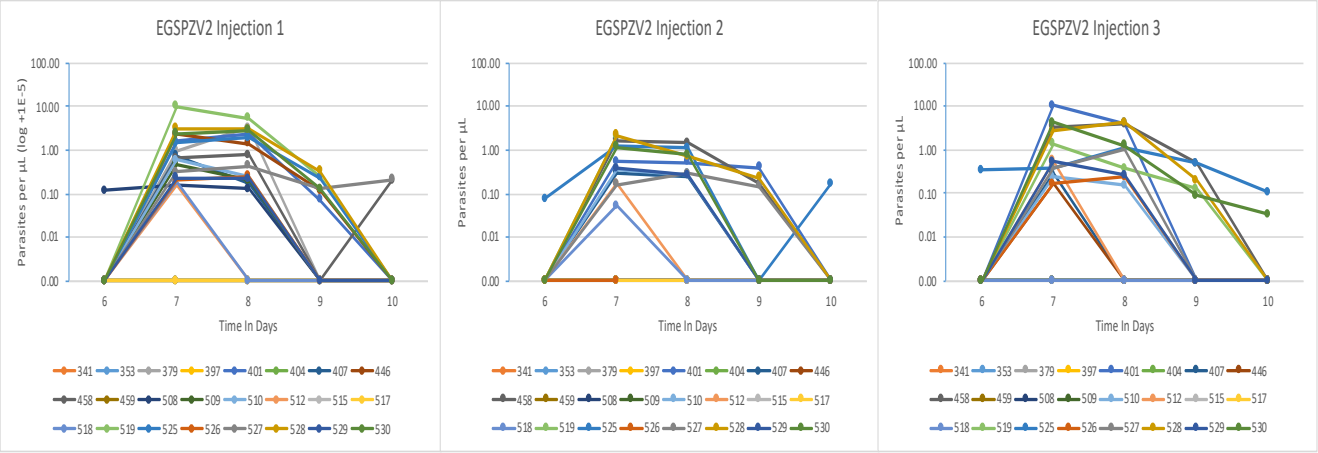


Figure 4: Parasite densities for all PfSPZ-CVac-immunized individuals in the EGSPZV2 trial. In each panel, PfSPZ Challenge was injected by DVI on day 0. Daily qPCR assays of parasite density were initiated on day 6. The lower limit of detection (LOD) was 0.020 parasites/µL. The volume of blood processed for detection of nucleic acid was 180 uL. Any undetected parasitemias are arbitrarily denoted as zero. No additional treatment beyond the prescribed partner drug (CQ) was administered and no volunteers included had recrudescent parasitemias. Note that several volunteers had a positive signal on day 10 or later, but these did not appear to be viable parasites as all study subjects became negative and no parasitemias recrudesced.

Summary of PfSPZ-CVac (chloroquine) safety concerns: In summary, PfSPZ-CVac (chloroquine) safety data indicate that malaria-naive adults, depending on the dose of PfSPZ

Challenge administered, may experience self-limited, Grade 3 AEs during the period of transient parasitemia at doses on the order of $2.0x10^5$ PfSPZ. However, initial experience from the ongoing PfSPZ-CVac (pyrimethamine) trial at NIH (protocol 17-I-0067) indicated that the Grade 3 AEs can be completely prevented by administration of an anti-pyretic/anti-inflammatory beginning on the 7th day after administration of PfSPZ Challenge. In contrast, malaria-exposed Africans with naturally acquired immunity experience, at most, mild (Grade 1) AEs, and these occur in a minority of individuals, and the rates of these AEs was similar to that of NS controls in the Equatorial Guinea trial, with the same result likely in Mali when the study is unblinded, since the rate of AEs in the combined vaccine and NS groups was very low.

The reason for the difference in tolerability profiles between malaria-naive and malaria-exposed individuals is almost certainly naturally acquired immunity, which is known to include anti-toxic functionalities that reduce the symptomatology associated with a given density of parasitemia (Schofield and Mueller 2006, Doolan, Dobano et al. 2009), although the mechanisms for this clinical tolerance are not yet identified. Naturally acquired immunity also appears to reduce the density of parasitemia by an undetermined mechanisms, which could be neutralizing PfSPZ, developing liver stage parasites, and/or developing blood stages. Evidence for reduced parasite densities in those with naturally acquired immunity compared to malaria-naive individuals comes from the EGSPZV2 trial, where parasite densities were ~10 times lower than in TÜCHMI-002 despite a 2-fold higher inoculum with PfSPZ Challenge.

The plan to increase PfSPZ Challenge doses for the PfSPZ-CVac (chloroquine) groups in the current study to $4.0x10^5$, twice the dose that appeared to be excellently tolerated in Mali, appears reasonable at this stage and is likely to be as well tolerated as lower doses. We do anticipate some cases of chloroquine induced, self-limited pruritus, as has been seen in Equatorial Guinea, but the incidence should be low, since pruritus is more likely to occur after therapeutic doses of chloroquine than the lower chemoprophylactic doses used during PfSPZ-CVac immunization (Gama, Ismael et al. 2009). Other CQ-induced side effects such as dizziness, blurred vision and tinnitus may also occur.

1.4.2.3 PfSPZ-CVac (chloroquine) and PfSPZ-CVac (pyrimethamine) in Malaria Naïve Populations

As summarized in **Section 1.3**, following the initial trial of PfSPZ-CVac (pyrimethamine) where the vaccine was safe but not sufficiently protective, a second open label, single center, phase 1 study has been undertaken at the NIH Clinical Center starting in 2017 (NIAID protocol #17-I-0067, ClinicalTrials.gov Identifier: NCT03083847). This is a phase 1 dose escalation study to investigate the safety and tolerability of PfSPZ-CVac (pyrimethamine) and PfSPZ-CVac (chloroquine) with PfSPZ administered by DVI. The study was designed to explore the immunogenicity and protective efficacy of this regimen, given as a 3-dose series, against homologous Plasmodium falciparum, NF54 (PfNF54) and heterologous (Pf7G8) CHMI. As stated in **Section 1.3**, the study has generated preliminary data showing the PfSPZ-CVac (pyrimethamine) using doses of $2.0x10^5$ PfSPZ of PfSPZ Challenge with each immunization can provide high level protection against heterologous CHMI conducted 12-13 weeks after immunization. This provides the core rationale for testing PfSPZ-CVac (pyrimethamine) in Mali under the current protocol. Safety data from the 17-I-0067 trial are presented here.

The study is divided into a pilot phase and a main phase. The pilot phase used small cohorts (n=2 for each group) to study whether the sequential increase of the dose of PfSPZ Challenge from $5.0x10^4$ to a target dose of $2.0x10^5$ SPZ while on either chloroquine or PYR is safe (in

particular, no breakthrough parasitemia in the PYR group) and well tolerated (in particular, the transient parasitemia that occurs in the chloroquine group is well tolerated). For the PYR pilot (*Arm 1x*), participants received 2 doses of PYR (50 mg), administered on 2 and 3 days post PfSPZ Challenge. For the chloroquine pilot (*Arm 5x*), participants received 2 doses of chloroquine, a loading dose (1000 mg salt) 1-2 days before PfSPZ Challenge and a second dose (500 mg salt) 5 days post PfSPZ Challenge. The pilot phase participants received only one PfSPZ Challenge injection and were not enrolled in the main study and did not undergo CHMI.

In the pyrimethamine pilot arms (n=8), all doses from $5.0x10^4$ to $2.0x10^5$ PfSPZ of Challenge SPZ were well tolerated. Most of the observed AEs were Grade 1 and many unlikely related to study participation. There was one transient Grade 3 AE of heat exhaustion in a participant who received the intermediate dose of $1.0x10^5$ PfSPZ of PfSPZ Challenge that was not related to study participation. A second Grade 3 AE of costochondritis, unrelated to study participation, occurred in a subject who received $2.0x10^5$ PfSPZ of PfSPZ Challenge. All AEs resolved without sequelae. Safety labs were also followed closely per protocol; there was one Grade 1 white count decrease and one Grade 1 ALT increase observed in one asymptomatic subject who received $2.0x10^5$ PfSPZ of PfSPZ Challenge. The WBC decrease and ALT increase have resolved without sequelae (**Table 10**).

Table 10. Summary of AEs by severity and frequency in pyrimethamine pilot study

PfSPZ Challenge with PYR	Grade 1	Grade 2	Grade 3	Grade 4	Total AEs
Arm 1a ; 50000 (n=2)	4 (2) 100%	0 (0) 0%	0 (0) 0%	0 (0) 0%	4 (2) 100%
Arm 1b; 100000 (n=2)	9 (2) 100%	0 (0) 0%	1 (1) 50%	0 (0) 0%	10 (2) 100%
Arm 1d; 200000 (n= 4)	8 (3) 75%	1 (1) 25%	1 (1) 25%	0 (0) 0%	10 (3) 100%

Note: X (X) X% refers to absolute number of AEs (Number of subjects with AEs) Percent of subjects with AEs

In the chloroquine pilot arms (n=6), overall, there were more reported AEs and related AEs compared to the PYR pilot arms. However, the majority were Grade 1 expected AEs (**Table 11**). At the dose of $1.0x10^5$ PfSPZ of PfSPZ Challenge, neither participant had a positive blood smear (0/2). At the dose of $2.0x10^5$ PfSPZ of PfSPZ Challenge, the majority of individuals (3/4; 75%) had a single positive blood smear with associated expected symptoms related to induced malaria, some of which were Grade 3 (fever and myalgia) in severity. Symptoms improved quickly or resolved with administration of 1 dose of NSAIDs (ibuprofen or acetaminophen). All subjects recovered and symptoms were at Grade 3 for less than 24 hours during the period of expected peak parasitemia. All chloroquine arm subjects were LMIV research qPCR positive for at least 2 consecutive days (day 7 and 8 post PfSPZ Challenge) as expected, with those experiencing the highest peak parasitemia being blood smear positive with associated Grade 3 symptoms. All participants were treated with Malarone® per protocol and completed their study participation per protocol. The summary of the AEs is outlined below (**Table 11**).

Table 11. Summary of AEs by severity and frequency observed in chloroquine pilot study

PfSPZ Challenge with CQ	Grade 1	Grade 2	Grade 3	Grade 4	Total AEs
Arm 5a ; 100000 (n=2)	16 (2) 100%	1 (1) 50%	0 (0) 0%	0 (0) 0%	17 (2) 100%
Arm 5b ; 200,000 (n= 4)	19 (4) 100%	5 (2) 50%	3 (2) 50%	0 (0) 0%	27 (4) 100%

Note: X (X) X% refers to absolute number of AEs (Number of subjects with AEs) Percent of subjects with AEs.

The results of the pilot study were reviewed by the Safety Monitoring Committee (SMC) which recommended continuing on to the main study at the doses of $2.0x10^5$ PfSPZ for both PfSPZ-CVac (pyrimethamine) and PfSPZ-CVac (chloroquine). However, the SMC recommended careful monitoring of Arm 3 (chloroquine arm) on the days of peak parasitemia and also the presumptive administration of NSAIDs on day 7-8 after each immunization, to help prevent Grade 2 and Grade 3 AEs. This intervention was added to the protocol as recommended by the SMC.

1.4.2.3.1 Main Phase

The main phase of this ongoing study is designed to assess the safety, tolerability and protective efficacy against CHMI of PfSPZ-CVac (pyrimethamine) and PfSPZ-CVac (chloroquine), using the maximum tolerated dose from the pilot trial, which for both vaccines was $2.0x10^5$ PfSPZ. The main phase was divided into two cohorts, and at this time, only the first cohort, comprising approximately half of the planned participants, has been completed.

In the pyrimethamine arms, *Arms 2a* and *2b,* (n=13) participants received three monthly injections of $2.0x10^5$ PfSPZ of PfSPZ Challenge. There have been a total of 104 AEs; most of the AEs, 97/104 (93%) were Grade 1. The most commonly reported AEs were fatigue, palpitations, arthralgia, and myalgia. There were four Grade 2 AEs (myalgia, gastroenteritis, Cr increase and hemoglobin decrease). There were 2 Grade 3 AEs, a gastroenteritis that was deemed not related to the study and a neutrophil decrease that was considered related to the study.

One participant reported three separate episodes of gastroenteritis that were all attributed to food that was consumed shortly before the symptoms began and not considered related to study products or procedures. One of the episodes was assessed as Grade 3 and one as Grade 2. Another participant reported a Grade 2 myalgia in her upper back after lifting a baby, six days after the third vaccination, not related to study products or procedures.

Three participants reported one or more episodes of Grade 1 palpitations. Two of the participants experienced multiple, brief episodes of isolated palpitations that began within a few hours of PYR dosing. Both participants denied chest pain, shortness of breath or diaphoresis. These episodes were assessed by the investigator as possibly related to both PYR and PfSPZ Challenge due to the timing of onset. Cardiac monitoring, electrocardiogram and holter monitoring, did not reveal any abnormalities. A third participant experienced intermittent palpitations beginning 9 days after PfSPZ Challenge inoculation that persisted for several days associated with anxiety and evaluation by cardiology did not reveal any cardiac abnormalities.

There was one reported episode of mild non-cardiac chest pain that occurred two days post 3rd PfSPZ Challenge injection and 1 dose of PYR, beginning approximately 12 hours after receiving PYR and lasting for approximately 4 hours.

Laboratory abnormalities have mostly been Grade 1. One participant had a Grade 2 decrease in hemoglobin deemed related to PfSPZ Challenge and/or PYR that occurred three days after PfSPZ Challenge injection, and a Grade 3 decrease in neutrophils that was also deemed related to PfSPZ Challenge and/or PYR that occurred three days after PfSPZ Challenge injection. This participant had pre-existing Grade 1 anemia and Grade 1-2 neutropenia. Another participant had a Grade 2 increase in serum Cr that occurred three days after PfSPZ Challenge injection. This participant had a pre-existing Grade 1 elevation in Cr. All abnormalities resolved within a few days without sequelae.

The development of parasitemia was monitored by the NIH Malaria qPCR assay, which has a lower limit of detection of approximately 500 parasites/mL. qPCR was performed daily from days 6-14 post PfSPZ Challenge after first vaccination and from days 7-9 post PfSPZ Challenge after the 2nd and 3rd vaccinations. Participants were monitored with blood smears on days 6-10 after the 1st vaccination, and on days 6 and 10 after the 2nd and 3rd vaccinations. All NIH malaria qPCRs were performed real time for safety follow up. All results were negative after immunization with PfSPZ-CVac (pyrimethamine). A second, more sensitive, RNA-based qPCR assay, the LMIV qPCR assay, with a lower limit of detection of approximately 20 parasites/mL, was performed retrospectively at the same time points, and confirmed that every timepoint was indeed negative (**Figure 5** below).

In the chloroquine arm, *Arm 3* (n=5), based on the results of the Pilot Phase and the recommendations of the SMC, NSAIDs were administered presumptively on expected peak parasitemia days to improve tolerability on days 7 and 8 post-vaccination #1. Presumptive NSAIDs were not administered after vaccination #2 and #3. 50 AEs were reported, the majority (96%) were Grade 1, expected AEs. We thus observed decreased severity of AEs after vaccination #1 in the main phase compared to the chloroquine arms in the pilot phase of the study, likely due to addition of presumptive NSAID use (**Table 12**).

The most commonly reported AE in the chloroquine Arm across all 3 injections was headache. Other commonly reported AEs include fatigue, tachycardia, arthralgia, and myalgia which are all expected AEs in the setting of malaria parasitemia. Most reported AEs were Grade 1. There were two unrelated Grade 3 SAEs described briefly below, and no Grade 2 AEs. Of note, there were fewer AEs reported with each subsequent injection.

Laboratory AEs reported included one episode of decreased WBC count, three episodes of decreased absolute lymphocyte count (ALC) (in two different participants), and two episodes of decreased hemoglobin (both in the same participant), all of which were Grade 1.

Table 12. Addition of NSAIDS improves tolerability of PfSPZ CVac (chloroquine)

PfSPZ Challenge with chloroquine	Grade 1	Grade 2	Grade 3	Total
Pilot *Arm 5b*, NO NSAIDS	19 (4) 100%	5(2) 50%	3 (2) 50%	27 (4) 100%
Main *Arm 3,* + NSAIDS	23 (5) 100%	0 (0) 0%	0 (0) 0%	23 (5) 100%

Note: *X (X) X% refers to the absolute number of AEs (number of subjects with AEs) percent of subjects with AEs*

Thick blood smears were completed daily from days 6-12 post PfSPZ Challenge. One blood smear was positive in one participant on day 8 after the first immunization with PfSPZ Challenge. Otherwise, all blood smears remained negative throughout. All participants had

subpatent parasitemia detectable by qPCR after the first PfSPZ Challenge administration as expected (**Figure 5**), although fewer subjects had detectable subpatent parasitemia with subsequent PfSPZ Challenge administrations, likely reflecting developing immunity. The decreasing frequency and density of parasitemia in second and third immunizations was expected as it has been a consistent finding in similar studies at LMIV and other centers.

Two participants were withdrawn from the study due to SAEs. One participant experienced acute change in mental status that required hospitalization due to acute anticholinergic poisoning after ingesting the seeds of a *Datura stramonium* plant (also known as Jimson weed) for recreational use. This episode that occurred after vaccination #2 was determined to be possibly related to chloroquine due to the the possibility that it may have interacted with the toxins in the Datura plant. A second SAE occurred after vaccination #3 in a participant who was hospitalized after developing a spontaneous pneumothorax that required chest tube placement. The participant recovered completely and was discharged after 3 days. Further evaluation during the hospital admission showed radiographic evidence of COPD. The SAE was assessed as unrelated to PfSPZ Challenge or chloroquine. Both participants recovered fully and were withdrawn from the study prior to CHMI.

Figure 5. Parasitemia curves in the pyrimethamine and chloroquine groups

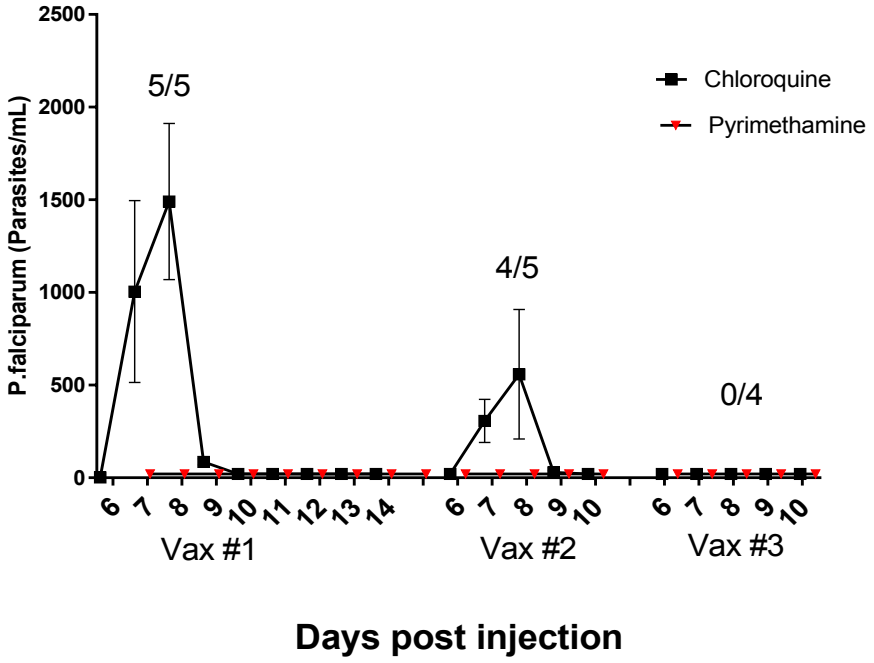


Figure 5. *(1) Pyrimethamine given at 2 and 3 days post injection completely prevented blood stage parasitemia. (2) The expected parasitemia in the chloroquine groups diminished rapidly with progressive immunizations.*

1.5 Clinical Trial Plan

Based on the promising preliminary results observed in the NIAID protocol 17-I-0067 described above, we plan to conduct a clinical trial in malaria-endemic regions of Mali to optimize and confirm safety, tolerability and development of protective immunity of new PfSPZ-CVac regimens in malaria-exposed adults against natural malaria transmission. We will investigate

immunological parameters including humoral and cell-mediated responses to identify potential mechanisms of protection, and to explain differences in VE between PfSPZ-CVac regimens comparing when blood stage exposure is present (chloroquine arms) to when blood stage exposure may be absent (pyrimethamine arms).

1.5.1 Rationale for a higher, one dose regimen of pyrimethamine

In the above described studies, in the PYR arms, participants have been dosed with 50 mg on 2 and 3 days post injection with PfSPZ Challenge. This PYR regimen was sufficient in arresting parasite development in the liver as assessed by qPCR (**Figure 5**). Pharmacokinetics studies were conducted in the first LMIV PfSPZ-CVac (pyrimethamine) study (#15-I-0169; NCT02511054) described above. Samples to determine serum PYR levels were collected on days 6, 10 and 26 (4, 8 and 24 days post first dose of PYR) post vaccinations #1 and 2 and on days 6, 10 and 84 post vaccination #3. The analysis showed that PYR mean concentrations remained above the *in vitro* IC50 (2.74 ng/mL) for PfSPZ Challenge NF54 (**Figure 6**). This data suggests that if PYR is dosed earlier, 2 days earlier and on the same day as PfSPZ Challenge injection which is a more practical regimen, the serum levels should remain above therapeutic level and PYR should be effective in killing the parasites in the liver. In addition, in planning for a broader use of this PfSPZ-CVac (pyrimethamine) vaccine regimen such as incorporating with seasonal malaria chemoprophylaxis and intermittent preventive treatment in pregnancy (IPTp) in which adults receive an equivalent of 75mg of PYR per dose, the proposed study will also use one dose of 75 mg of PYR. From the available pharmacokinetic data, we can extrapolate that the achieved serum levels at this regimen will be sufficient to completely prevent the emergence of subpatent parasitemia.

Figure 6: Serum pyrimethamine levels in PfSPZ-CVac (pyrimethamine) study NCT02511054 in malaria-naïve individuals

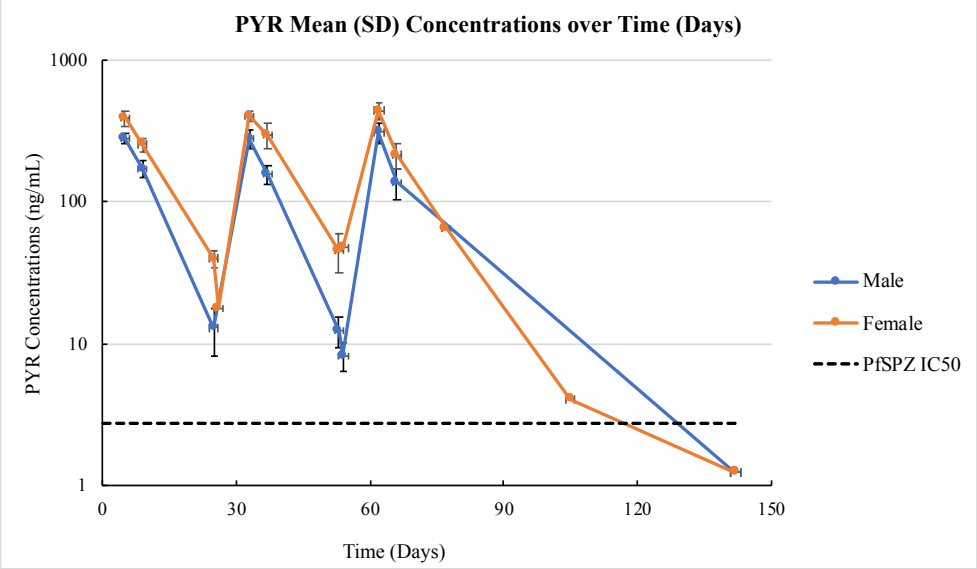


Figure 6: 50 mg of pyrimethamine was administered on days 2 and 3 post PfSPZ Challenge NF54 DVI. Blood samples were collected 4, 8 and 24 after the first dose of pyrimethamine at each vaccination #1 and #2. During vaccination #3, blood samples were collected on 4, 8 and 82 days post the first dose of pyrimethamine. **Note** X-axis is time post the first dose of pyrimethamine.

1.5.1 Rationale for administering a booster dose

The main phase of the study was designed to assess the safety and immunogenicity of three monthly doses of PfSPZ-CVac (PYR) or PfSPZ-CVac (CQ) with follow-up over an initial malaria transmission season. Eligible participants on the main phase of the study will be offered a booster dose to extend the study through a second malaria transmission season.

A previous study of PfSPZ Vaccine (ClinicalTrials.gov Identifier NCT03510481) conducted by LMIV in Mali in recent years also employed a booster vaccination. However, there have been no booster vaccinations given in an area of endemic malaria transmission after a primary series of PfSPZ CVac (PYR) or PFSPZ CVac (CQ) in the past. Administering a booster dose approximately 11 months after the initial course of vaccinations on this study would allow for an assessment of safety, immunogenicity, and efficacy of over a second malaria transmission season, which has never been performed before for this category of malaria vaccine. Understanding the role of boosting for this vaccine approach is essential to determine whether endemic populations who have received a primary vaccination series could benefit from receiving a booster dose prior to each transmission season rather than a repeated primary series of vaccine.

1.5.1.1 Safety

The study began screening participants in May 2019. The pilot phase consisted of 12 participants who received $4.0x10^5$ PfSPZ of PfSPZ Challenge on 23 May 2019 plus a partner drug of either CQ dosed on days -2 and -5 or PYR dosed on day 0 or on days +2 and +3. All study medications and vaccinations were safe and very well tolerated with no evidence of breakthrough parasitemia by either sensitive blood smear or LMIV qPCR.

The main phase began recruiting in May 2019 and enrolled 249 participants, of whom 240 were randomized into either Arm 1b/4a or Arm 2b/4b and received at least one vaccination. All vaccinations for Arms 1b/4a and 2b/4b were completed as of 23 September 2019. Currently (March 2020), participants in Arms 1b/4a and 2b/4b are nearing the end of the malaria follow-up phase during the first transmission season. Overall the study medications and vaccinations were safe and very well tolerated as summarized below.

Arm 1b/4a: PfSPZ-CVac (PYR) or normal saline with PYR administered on day 0 (n=144)

144 participants of a projected 144 received the first vaccination (or NS injection), with 140 receiving the second and 139 receiving the third. All participants were given artemether/lumefantrine approximately 7-14 days prior to first and third vaccinations per protocol. All participants that remain on study are in the NHMI monitoring phase and have completed follow up visits through at least study day 224.

There have been a total of 577 AEs to date. 231 (41%) were Grade 1, 339 (58%) were Grade 2, and 7 (1%) were Grade 3. The most common AEs have been rhinitis (0/79 deemed related to study procedures, 72/79 Grade 2), elevated serum creatinine (50/65 related to study procedures, 6/65 Grade 2 of which 5 were related to study procedures), decreased leukocyte count (38/63

related to study procedures, 5/63 Grade 2 of which 3 were related to study procedures), and decreased ANC count (32/52 related to study procedures, 11/52 Grade 2 of which 7 were related to study procedures, and 2/52 Grade 3 of which both were related to study procedures).

To date, there have been 7 Grade 3 AEs reported, of which the only two that were related to study procedures were the two episodes of neutropenia noted above. In addition, there have been three episodes of elevated blood pressure, 1 episode of paronychia, and one episode of intestinal volvulus that was also an SAE (described in more detail below). There have been no Grade 4 or Grade 5 AEs.

Local site reactogenicity occurred in 3 participants (2%) including 1 episode of Grade 2 injection site pain. Solicited systemic AEs occurred in 13 participants (9%), 6 of which were Grade 2 (most commonly headaches).

SAE:

There was one SAE that occurred in this arm. On 20 August 2019, participant CV-19-877-D presented to the study clinic with acute abdominal pain and was subsequently admitted to the referral hospital at Point G, Bamako and diagnosed with a sigmoid volvulus by ultrasound. The volvulus resolved with noninvasive medical management and the participant was discharged after two days. On follow-up visits at the study clinic he continued to improve with resolution of all symptoms. The volvulus was determined unlikely to be related to study procedures. The sponsor, DSMB and site safety monitor all agreed the participant could continue on study if he chose to. He continued on the study with no further issues other than an episode of acute malaria contracted during the natural human malaria infection (NHMI) phase.

Arm 2b/4b: PfSPZ-CVac (PYR) or normal saline with PYR administered on days 2 and 3 (n=96)

96 participants of a projected 96 received the first vaccination (or NS injection), with 95 receiving the second and 93 receiving the third. All participants were given artemether/lumefantrine approximately 7-14 days prior to first and third vaccinations per protocol. All participants that remain on study are now in the NHMI monitoring phase and have completed follow up visits through at least study day 224.

There have been a total of 424 AEs to date. 171 (42%) were Grade 1, 249 (57%) were Grade 2, and 4 (1%) were Grade 3. The most common AEs have been rhinitis (0/63 deemed related to study procedures, 55/60 Grade 2), elevated serum creatinine (45/56 related to study procedures, 9/56 Grade 2 of which 2 were related to study procedures), and decreased leukocyte count (36/55 related to study procedures, 2/55 Grade 2 of which 1 was related to study procedures), decreased ANC count (19/40 related to study procedures, 10/40 Grade 2 of which 5 were related to study procedures, and 1/40 Grade 3 which was related to study procedures).

Local site reactogenicity occurred in 3 participants (3%), all of which were mild (Grade 1). Solicited systemic AEs occurred in 22 participants (22%), 13 of which were Grade 2 (most commonly headaches).

To date, there have been 4 Grade 3 AEs reported, of which one episode of neutropenia as noted above was related to study procedures. In addition, there was one episode of joint dislocation not requiring hospitalization and not related to study procedures. There have been no Grade 4 or Grade 5 AEs or SAEs.

1.5.1.2 Malaria infection rate

Vaccine efficacy monitoring began after the third vaccination. Participants have been evaluated for malaria infection using blood smears every two weeks. The study is currently still blinded.

Table 13: Parasitemia summary for main phase Arms 1b/4a and 2b/4b by calendar month post third vaccination as of 27 March 2020

	Total + Pf BS post third vaccination	**Pf + BS in unique individuals**	**% Unique individuals with Pf + BS**
September 2019	21	15	6.4%
October 2019	79	58	25.0%
November 2019	74	29	12.5%
December 2019	26	7	3.0%
January 2020	8	1	0.4%
February 2020	9	2	0.9%
Total	**217**	**112**	**48.2%**

BS = blood smear; Pf = *Plasmodium falciparum*

1.5.1.3 Futility criteria

An analysis of safety and of vaccine efficacy through the NHMI phase following the primary vaccination series will be conducted after all originally-planned primary and secondary endpoints from these objectives have been gathered. The booster phase will proceed as planned unless both Arm 1b or Arm 2b fail to demonstrate statistically significant vaccine efficacy for the secondary endpoint, or unless the power is judged to be insufficient with the available numbers of participants.

If one of the two PYR vaccination arms (Arm 1b or Arm 2b) meets all of the above criteria but the other does not, then a booster vaccination dose will be given in the qualifying arm (and NS given to its corresponding control arm) while NS will be administered to both the vaccination arm that failed to qualify and to its corresponding control arm. This approach is meant to preserve the blinding of the study and to maximize the pooled control groups during the booster phase.

To assess if the power is sufficient to move forward with the booster phase, an analysis will be undertaken by the unblinded study statistician to perform sample size calculations for the booster phase. The study will proceed unless the power to detect a vaccine efficacy of either 50% by time-to-event analysis or 35% by proportional analysis, using the number of participants eligible for the booster vaccination minus 10% (to allow for loss to follow-up), is below 70%.

The study team will remain blinded to the analyses and the study statistician will communicate the results to the study team in a binary yes/no manner to either proceed or not with the booster dose. If the booster phase does not proceed in either PYR arm for any reason, unblinding and statistical analysis may proceed.

The same approach will be applied to the CQ group (Arms 3b/4c) at the appropriate time.

In addition, group unblinding for statistical analysis of blood smear results from the first year of the study (prior to the booster dose) will occur approximately 4 months following receipt of the booster vaccine dose, as long as individual subject blinding can be maintained (as determined by the unblinded statistician). The data will be reviewed by study staff who have no direct contact with study participants nor conduct clinical assessments. This information will only be used to determine if enough data supports moving forward with the pyrimethamine comparator arms on an accelerated vaccination schedule in a future planned trial.

1.5.2 Study Plan

The design of the proposed clinical trial entails 3 phases, a pilot phase, a main phase, and a booster phase:

1) **Pilot trial phase** is divided in two additional parts, PYR and chloroquine phases:
   a) PYR only (*Arms 1a, 2a, and if needed 5a, 6a*): the pilot will simultaneously examine whether increasing the dose of PfSPZ Challenge to $4.0x10^5$ PfSPZ with an increased dose of PYR to 75 mg given concurrently (day 0 only, *Arm 1a*) OR on day 2 &3 (*Arm 2a*) is safe and does not result in patent parasitemia (sensitive blood smear [sBS] negative in 4 of 4 subjects in each *Arm*). If there is patent parasitemia in either *Arms 1a or 2a*, the dose of PfSPZ Challenge will be decreased to $3.0x10^5$ PfSPZ in *Arms 5a* (PYR on day 0) or *6a* (PYR on day 2 &3) respectively, the dose of PYR will be maintained. If breakthrough parasitemia occurs in *Arm 5a* or *6a* the study will pause for reassessment.
   b) Chloroquine only Arm (*Arm 3a*): to assess safety and to ensure that the increased dose of $4.0x10^5$ SPZ while on weekly chloroquine regimen is tolerable (will not result in parasitemia related Grade 3 AEs lasting more than 48 hours despite adequate management). If symptoms are deemed intolerable, NSAIDs will be added to the regimen in the main phase of the study.(*Arm 3b*).

2) **Main trial phase and booster phase** will assess the safety, tolerability and VE of PfSPZ-CVac (pyrimethamine)
   a) *Arm 1b/5b*: $4.0x10^5$ /$3.0x10^5$ PfSPZ with 75 mg PYR given on days 0 (concurrent administration)
   b) *Arm 2b/6b*: $4.0x10^5$ /$3.0x10^5$ PfSPZ with 75 mg PYR given on days 2&3 post DVI

The main trial phase and booster phase will also assess the safety, tolerability and VE of PfSPZ-CVac (chloroquine)

a) *Arm 3b*: 4.0x10^5 PfSPZ +weekly chloroquine prophylaxis +/- NSAIDs dose level. Presumptive administration of NSAIDs on days 7 and 8 after the first vaccination only will be added if required as determined in the pilot phase.

The main phase and booster phase will also enroll placebo control arms that will undergo injections with NS and receive either PYR or chloroquine.

a) *Arm 4a:* Normal saline injection with 75 mg PYR given on days 0
b) *Arm 4b:* Normal saline injection with 75 mg PYR given on days 2 and 3
c) *Arm 4c:* Normal saline injection while on weekly chloroquine chemoprophylaxis

VE will be measured against naturally transmitted *P. falciparum* infection. Participants in all three arms of the main study will receive three successive immunizations at 4 week intervals followed by assessment of parasitemia every two weeks by thick blood smears during the transmission season beginning 2 weeks post the 3rd immunization. Participants will also receive a 4th (booster) immunization at approximately 11 months after the third vaccination followed by assessment of parasitemia every two weeks by thick blood smears during the transmission season beginning 2 weeks post the 4th immunization

All participants in the main phase will undergo parasitemia clearance with artemether/lumefantrine treatment twice during the course of the study, approximately 1-2 weeks prior to first and third vaccinations as outlined in **Appendix A**. In the pyrimethamine arms, the first dose of artemether/lumefantrine will be administered within approximately one week of the first vaccination where as in the chloroquine arms, this first dose will be administered within approximately two weeks of the loading dose of chloroquine. The first course of artemether/lumefantrine administered prior to the first vaccination aims at clearing naturally acquired malaria infection. In other similar studies conducted by LMIV in Mali, artemether/lumefantrine was administered to a total of 93 participants within approximately 4 days of first vaccination and there were no significant differences in safety measures between the vaccinated and unvaccinated participants and showed significant vaccine efficacy (Sissoko, Healy et al. 2017). One of the major concerns of concurrent administration of some antimalarials is prolongation of QTc (Traebert and Dumotier 2005).While pyrimethamine does not prolong QTc, chloroquine may prolong QTc (Haeusler, Chan et al. 2018). In the chloroquine groups, when a loading dose is administered prior to the first vaccination, approximately two weeks interval will be maintained between the administration of chloroquine and artemether lumefantrine, see **Appendix A**. Decreasing the interval between administration artemether/lumefantrine and pyrimethamine should not negatively impact the safety of the participants and is unlikely to affect the vaccine.

Participants in the booster phase will undergo one additional round of parasitemia clearance with artemether/lumefantrine approximately 1-2 weeks prior to fourth vaccinations as outlined in **Appendix A.**

Antibody and cellular immune responses and transcriptomic analyses will be assessed periodically after vaccination in order to identify correlates of protection**.**

2 Study Objectives

2.1 Primary Objectives

Safety

Pilot Phase

- To evaluate whether PYR on day 0 prevents patent parasitemia post DVI administration of $4x10^5$ PfSPZ or $3x10^5$ PfSPZ (if necessary) of PfSPZ Challenge (*Arms 1a, 2a, 5a, 6a).*
- To monitor the safety and tolerability of PfSPZ-CVac (chloroquine), post $4x10^5$ PfSPZ Challenge administered via DVI with chloroquine as the partner drug (*Arm 3a*)

Main Phase and Booster Phase

- To monitor the safety and tolerability of PfSPZ-CVac (pyrimethamine), PfSPZ Challenge administered via DVI with PYR as the partner drug; (*Arms 1b/5b, 2b/6b, 4a, 4b)*
- To monitor the safety and tolerability of PfSPZ-CVac (chloroquine), PfSPZ Challenge administered via DVI with chloroquine as the partner drug; (*Arms 3b, 4c*)

2.2 Secondary Objective

Protective Efficacy

- To assess the protective efficacy of PfSPZ-CVac (pyrimethamine) or PfSPZ-CVac (chloroquine) against natural *P. falciparum* infection (*Arm 1b/5b, 2b/6b, 3b, 4*).

2.3 Exploratory Objectives

Pyrimethamine regimen efficacy and Immunogenicity

- To evaluate whether PYR on day 0 or on day 2&3 prevents patent parasitemia post DVI administration of $4x10^5$ OR $3x10^5$ PfSPZ of PfSPZ Challenge (*Arms 1, 2, 5, 6).*
- To assess the humoral and cell mediated immune responses to PfSPZ and to known pre-erythrocytic and blood stage antigens *(All Arms)*
- To assess the humoral and cell mediated immune responses to novel pre-erythrocytic antigens *(All Arms)*
- To look for immune correlates of protection
- To assess the kinetics of subpatent parasitemia during PfSPZ-CVac immunization *(Arms 1, 2, 3, 5, 6)*
- To describe changes in γδ T cells in malaria experienced individuals after PfSPZ-CVac immunization and malaria infection during transmission season *(Arms 1b/5b, 2b/6b, 3b)*

3 Study Design

3.1 Overall Design

The study proposed here by MRTC/ University of Sciences, Techniques and Technologies of Bamako (USTTB), Sanaria and LMIV will assess PfSPZ-CVac (pyrimethamine) and PfSPZ-CVac (chloroquine) in a malaria experienced population in Mali.
The design of the proposed clinical trial will entail three phases, a pilot phase and a main phase followed by a booster phase:

Pilot Phase

a) **Pyrimethamine Arms (*1a, 2a, 5a, 6a*):** to assess safety and tolerability of increasing PfSPZ Challenge dose to $4.0x10^5$ PfSPZ with either concurrent administration of PYR (day 0 post PfSPZ DVI, *Arm 1a*) or PYR administration on days 2 & 3 post DVI (*Arm 2a*). These pilot arms will assess that these regimens will not result in patent parasitemia (blood smear negative). If needed, following a predefined algorithm (see **Figure 7** and **Figure 8**), PYR, the dose of PfSPZ Challenge will be decreased to $3.0x10^5$ PfSPZin *Arms 5a* and *6a*.

b) **Chloroquine Arms (*Arms 3a*):** to assess safety and tolerability of PfSPZ Challenge ($4.0x10^5$ PfSPZ) and chloroquine dosing on day -2 and +5 and to assure that these regimens will not result in Grade 3 AEs that last more than 48 hours with adequate management during the expected period of parasitemia or require treatment with another antimalarial medication. If symptoms related to patent parasitemia are deemed intolerable, then subjects will receive presumptive treatment with non-steroidal anti-inflammatory drugs (NSAIDs) at the time that transient parasitemia develops following the first immunization and as needed thereafter in the main phase (see **Figure 8**).

Main Phase and Booster Phase

a) **Pyrimethamine Arms (*Arms 1b/5b, and 2b/6b*):** to assess safety and tolerability of 3 exposures every 28 days of $4x10^5$ PfSPZ and a booster exposure while under PYR given on either day 0 (*Arm 1b*) *or* day 2 & 3 (*Arm 2b*)post DVI . *Arms 5b* (PYR on day 0) or *6b* (PYR on day 2&3) that receive $3x10^5$ PfSPZ will only be enrolled if there is patent parasitemia with the higher dose of PfSPZ as determined by the pilot phase (**Figure 7**). The main phase will also assess VE against natural *P. falciparum* infection (see ***Figure 9***).

b) **Chloroquine Arm (*Arm 3b*):** to assess safety and tolerability of three exposures every 28 days to PfSPZ Challenge ($4.0x10^5$ PfSPZ) and chloroquine dosing on day -2, +5 post PfSPZ DVI and weekly thereafter (10 doses in total) and a booster exposure while under chloroquine dosing on day -2, +5 post PfSPZ DVI and weekly thereafter (2 doses in total) and VE against natural *P.falciparum* infection (see ***Figure 9***). NSAIDs may be administered on day 7 &8 post first vaccination, if required, as determined by the pilot phase.

c) **Placebo Control Arm (*Arm 4*):** to serve as controls enabling comparison during the assessment of safety, tolerability and VE of the vaccinated arms against natural

P.falciparum infection (see ***Figure 9***). Participants will receive 3 exposures of NS every 28 days while under PYR given on either day 0 *or* day 2 & 3 post vaccination (*Arm 4a* or *4b* respectively) or chloroquine dosing on day -2, +5 post injection and weekly thereafter for 10 doses in total (*Arm 4c*) plus a booster exposure of NS while either under PYR given on either day 0 *or* day 2 & 3 post vaccination (*Arm 4a* or *4b* respectively) or CQ dosing on day -2, +5 post injection and weekly thereafter for 2 doses.

3.2 Pilot Study

The overall goal of the pilot study is to assess the safety and prevention of patent parasitemia in concurrent administration of PYR as well as increasing dose of PfSPZ Challenge to $4.0x10^5$ PfSPZ under PYR as well as the safety and tolerability of administering $4.0x10^5$ PfSPZ under chloroquine prophylaxis. Participants in the pilot phase will be randomized into either *Arm 1a, 2a* or *3a*. If other pilot arms are required (*Arm 5a* or *6a*), enrollment will be sequential. **Note:** If enrollment into *Arms 5a* and *6a* occurs prior to completion of *Arms 1a, 2a* or *3a*, the participants will be randomized into either *Arm 5a* or *6a*. Appropriate regimens for further testing in the main phase will be selected from the pilot study results.

In the pyrimethamine pilot, subjects will undergo immunization with PfSPZ-CVac with one exposure to $4.0x10^5$ PfSPZ Challenge with 75 mg of PYR dosing on day 0 only (*Arm 1a*) or day 2&3 (*Arm 2a*) post PfSPZ Challenge (**Figure 7**). Participants will be followed with sBS conducted from day 6 through 12 post injection. The goal of this pilot is to determine that increasing the dose of PfSPZ with either standard dosing of PYR on day 2&3 (*Arm 2a*) and also earlier administration of one dose of PYR (day 0, *Arm 1a*) does not lead to patent parasitemia. Samples for assessment of subpatent parasitemia breakthrough by qPCR will also be collected from day 6 through day 12 post PfSPZ Challenge injection and will be run retrospectively. Administration of 50 mg of PYR on days 2 and 3 post $2.0x10^5$ PfSPZ DVI in healthy US adults prevented subpatent and patent parasitemia as assessed by qPCR and blood smears respectively (**Figure 5**). With known pharmacokinetics of PYR, it is expected that the proposed dose of PYR, 75mg, will be sufficient to prevent subpatent and patent parasitemia even with higher dose of PfSPZ (**Figure 6**). Enrollment in these arms will occur simultaneously. If there is patent parasitemia, the dose of PfSPZ will be decreased to $3.0x10^5$ PfSPZ in alternate *Arms*. If there is patent parasitemia in *Arm 1a*, four participants will be enrolled in *Arm 5a* to receive $3.0x10^5$ PfSPZ while under 75 mg of PYR on day 0. If there is patent parasitemia in *Arm 2a*, four participants will be enrolled in *Arm 6a* to receive $3.0x10^5$ PfSPZ while under 75 mg of PYR on days 2 and 3 post injection.

Note:

- Participants to be enrolled in either *Arm 5a* or *6a* may be enrolled and receive artemether/lumefantrine prior to the end of the follow up period of *Arm 1a* or *2a*. However, they will **not** receive PfSPZ Challenge injection until the follow up period for *Arms 1a* and *2a* is complete (study day 13).
- If participants in *Arms 5a* or *6a* do not receive PfSPZ Challenge injection, they will be offered enrollment in the main study

If the regimen in *Arm 1a* and *2a* are safe and there is no patent parasitemia, these regimens will continue to the main study. The regimens that result in patent parasitemia will not continue to

the main study. If *Arm 5a* and *6a* result in patent parasitemia, the study will pause for reassessment. Samples for assessment of subpatent parasitemia breakthrough by qPCR will be collected from day 6 through day 12 post PfSPZ Challenge injection and will be run retrospectively. Subjects will be treated with standard treatment doses of artemether/lumefantrine at the end of their enrollment, starting 12 days post injection. Subjects from the pilot study will not join the main study.

Figure 7. Pyrimethamine Pilot Study flowchart Schema

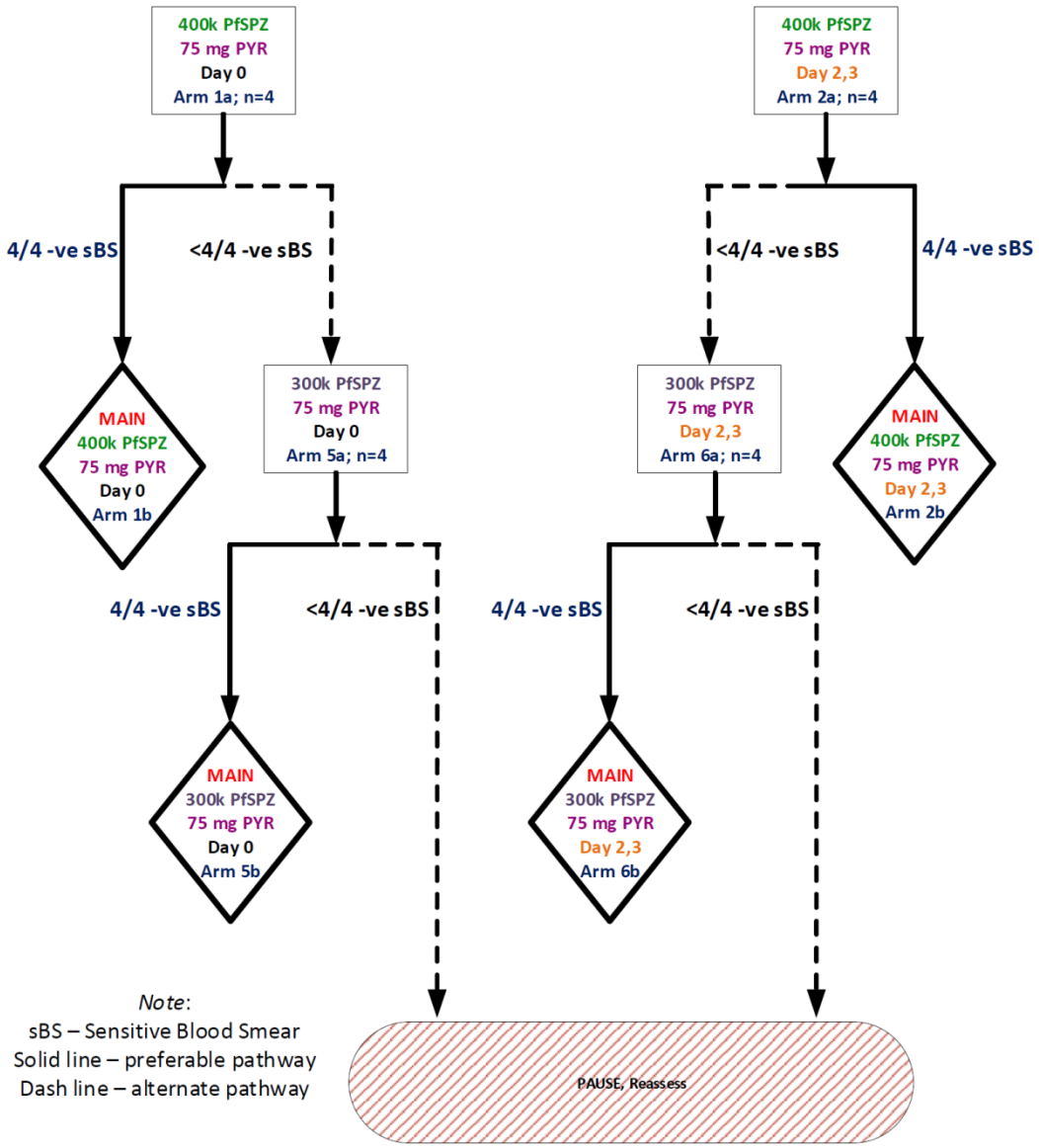
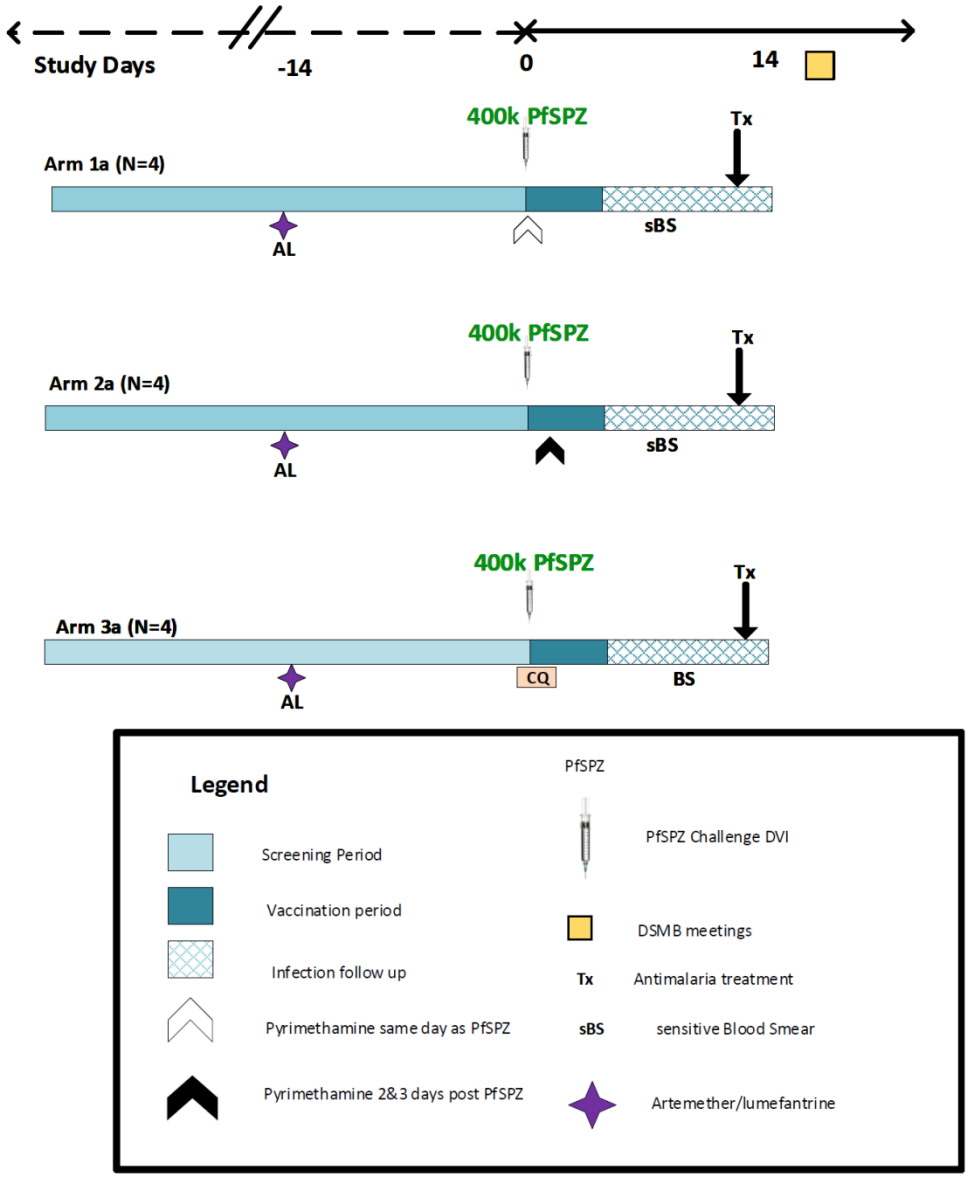


Figure 8. Ideal Pyrimethamine and Chloroquine Pilot Study Schema



In the chloroquine pilot study (*Arm 3a*), subjects will be immunized with PfSPZ-CVac with one exposure to PfSPZ Challenge, with a loading dose of chloroquine administered 2 days before PfSPZ Challenge and maintenance dose administered 5 days post PfSPZ Challenge (**Figure 8**). The goal of the pilot study is to evaluate safety and tolerability of administering a dose of 4.0×10^5 PfSPZ of PfSPZ Challenge. If this regimen is not tolerable, that is, it results in grade 3 or higher signs or symptoms lasting more than 48 hours despite adequate management, then NSAIDs will be administered on day 7 and 8 post PfSPZ Challenge after the first vaccination only in the main phase. In addition, all participants will be treated with full dose of artemether/lumefantrine at the end of the pilot, starting at day 12 post injection or if diagnosed with clinical malaria per Malian guidelines prior to day 12 days post injection. Participants from the pilot study will not join the main study.

3.3 **Main Study and Booster Phase**

The main phase, which will be conducted over several years, will be a randomized double blind placebo controlled study. Both the participants and investigators will not be aware of the injection assignment (PfSPZ Challenge or normal saline) but they will be aware of partner drug assignments, either PYR or CQ. In the vaccinated arms, participants will be enrolled in either:

- *Arm 1b/5b* ($4.0x10^5$ /$3.0x10^5$ PfSPZ + 75 mg PYR on day 0 post PfSPZ Challenge, n=90) *or*
- *Arm 2b/6b* ($4.0x10^5$ /$3.0x10^5$ PfSPZ + 75 mg PYR on days 2 & 3 post PfSPZ Challenge, n=60) *or*
- *Arm 3b* ($4.0x10^5$ PfSPZ + weekly chloroquine prophylaxis+/- NSAIDs, n=90)

In the control arms, 180 participants total will be enrolled. Participants will be enrolled in either

- *Arm 4a:* Normal saline injection with 75 mg PYR given on days 0 (concurrent administration, n=54) *or*
- *Arm 4b:* Normal saline injection with 75 mg PYR given on days 2 & 3, n=36 *or*
- *Arm 4c:* Normal saline injection while on weekly chloroquine chemoprophylaxis, n=90

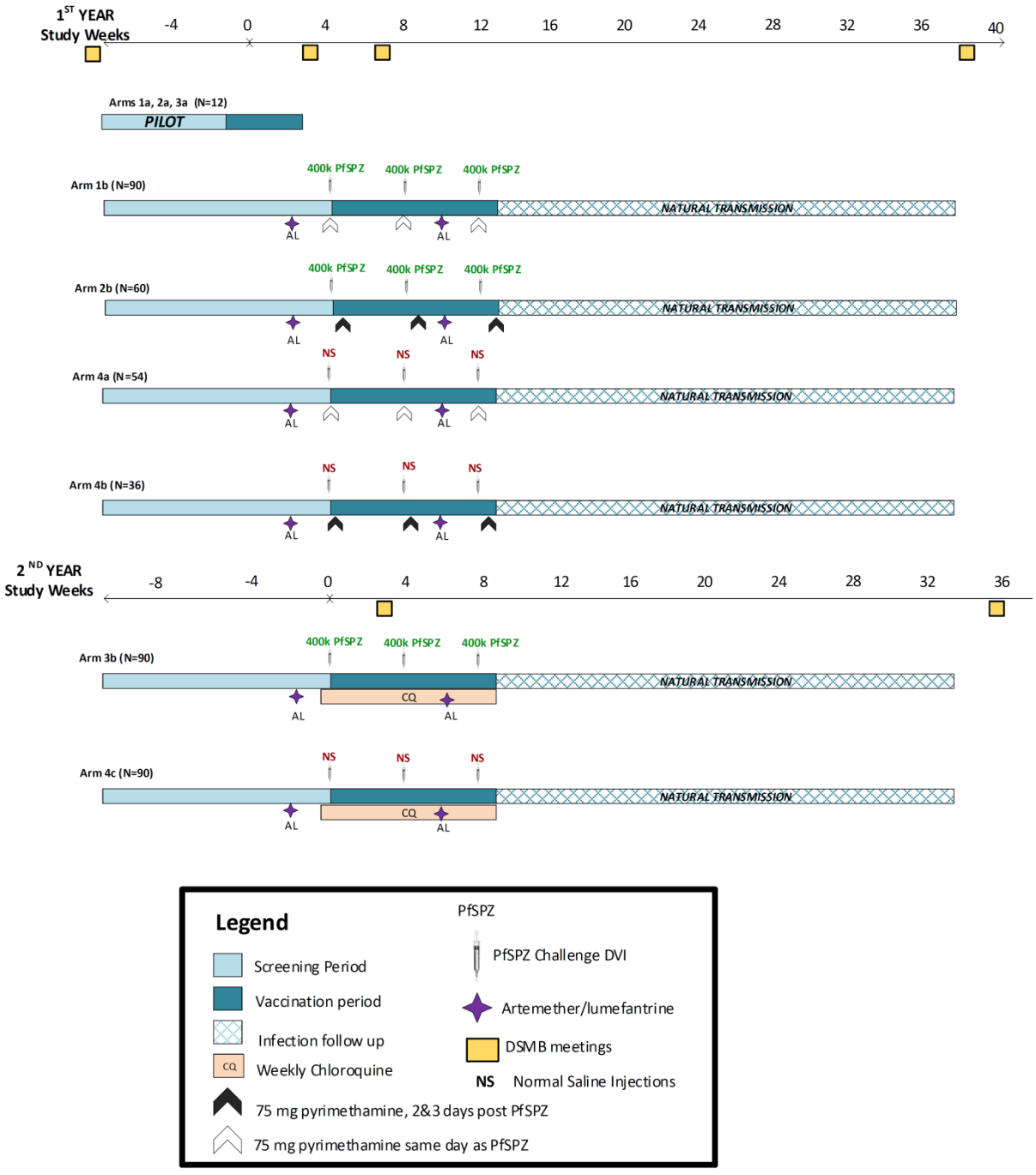
Note:
1. The number of participants in each of the control arms can be redistributed if one the planned vaccinated arms is not enrolled. The goal would be to have the total number of participants in the control arm to equal the number of participants in one of the vaccinated arms with the highest sample size.
2. The pyrimethamine arms will be enrolled in the first year and the chloroquine arms will be enrolled in a subsequent year of the study.

Participants in the vaccinated arms of the main study will receive three exposures to PfSPZ Challenge at 28-day intervals, with PYR or chloroquine coverage (PfSPZ-CVac,

Figure 9). The goal in the main study is to have two PYR regimens, the regimen of highest interest in which PYR is administered concurrently to PfSPZ Challenge (Arm 1b/5b) and a regimen in which a standard PYR dosing regimen is employed (*Arm 2b/6b*). Placebo control arms (*Arm 4*) will receive injections with NS following the same schedule as vaccinated arms while on PYR dosed either on day 0 *or* day 2 & 3 post vaccination (*Arm 4a* and *4b* respectively) or chloroquine dosing on day -2, +5 post injection and weekly thereafter (*Arm 4c*). The participants in the control *Arm 4*, will follow the same study procedures as those in vaccinated Arms thus providing blinding of the of the type of injection received (PfSPZ Challenge or NS) for the corresponding medication dosing vaccinated arms.

Participants in the main study (vaccinated and placebo controls) will receive two full doses of artemether/lumefantrine during the course of the study, approximately one to two weeks prior to the first and the 3rd injection. Administration of artemether/lumefantrine for the main phase can be started prior to completion of the pilot study. However, administration of chloroquine, pyrimethamine and PfSPZ Challenge will occur after completion of the pilot phase follow up (study Day 13). These participants will then be evaluated every two weeks for parasitemia during the malaria transmission season starting approximately 2 weeks from the third injection.

Figure 9. Main Study Schema



Booster Phase: At the completion of the follow up phase after the primary series, participants who received all three vaccine doses will be invited to continue participation in the study to receive a booster vaccine dose. This 4th dose will be administered prior to the beginning of the following transmission season, approximately 11 months post the 3rd vaccination. Those enrolled in the vaccine arms (*Arms 1* and *2*) will receive one injection of 9 x10^5 PfSPZ while those participants enrolled in the control arms (*Arms 3a* and *3b*) will receive one injection of normal saline. *Arms 1b, 2b,* and *3b* will each receive a single dose of 4x10^5 PfSPZ Challenge

whereas *Arms 4a, 4b,* and *4c* will receive normal saline while under their respective chemoprophylaxis regimens (PYR on day 0 for *Arms 1b/4a*, PYR on days 2 and 3 for *Arms 2b/4b*, and CQ on days -2 and 5 for *Arms 3b/4c*). Study blinding will be maintained as the participants transition from the main phase to the booster phase. The booster phase will extend malaria infection follow-up through the subsequent transmission season. If statistically significant protection is not obtained in both Arms 1b or 2b, the non-significant arm will be administered normal saline instead of PfSPZ Challenge as described in Section 1.5.1.3 (futility criteria).

4 Description of Investigational Products and Plan

4.1 Chloroquine Phosphate (CQ)

Chloroquine has been widely used for treatment and prophylaxis of malaria since 1946 (Most, London et al. 1984). It was the treatment of choice for uncomplicated malaria for decades because it was safe, well tolerated, affordable and highly effective for the treatment of malaria. However, increasing spread of chloroquine-resistant Pf over the last two decades has severely limited its use (Wellems 2002). CQ is U.S Food and Drug Administration (FDA) approved for suppressive treatment (prophylaxis) and for acute attacks of malaria due to *P. vivax, P. malariae, P. ovale,* and susceptible strains of Pf. Chloroquine is a blood-stage schizonticide, highly active against replicating forms of blood-stage drug-sensitive parasites. As such, it is routinely employed as first-line prophylaxis against development of patent parasitemia and clinical malaria in non-immune travelers to areas with chloroquine sensitive Pf. Under standard weekly dosing, as used in this study [1000 mg (600 mg base) loading dose then 500mg (300mg base) weekly], routine monitoring in clinical practice for patent blood-stage infection (via blood smear or qPCR) is not typically conducted unless triggered by presentation for evaluation of malaria related symptoms. Weight based chloroquine dosing also has been approved for treatment and prevention of Pf malaria.

The current approved package insert for chloroquine and additional details on safety of chloroquine used with PfSPZ Challenge are provided in the Investigator's Brochure (IB).

4.2 Pyrimethamine

Pyrimethamine is a folic acid antagonist that has been commonly used as antimalarial drug for both treatment and prevention of malaria, usually in combination with sulfadoxine in adults, pregnant women, and children worldwide (Organization April 2013 (rev. January 2014)). Similar to chloroquine, PYR (in combination with sulfa drugs) was a drug of choice for malaria treatment and prophylaxis for decades because it was safe, well tolerated, affordable and highly effective; however, due to widespread resistance, PYR and PYR combinations have fallen out of favor for clinical use. Since the 1950's, PYR has been FDA approved for acute treatment and chemoprophylaxis of malaria due to susceptible strains of plasmodia. Should circumstances arise, PYR may be used alone for acute malaria at 50 mg orally for two days. PYR has been shown to possess both tissue schizonticidal (i.e., liver stage specific) and blood schizonticidal activity against the malaria parasite *in vitro* and *in vivo* (Friesen, Borrmann et al. 2011, Delves, Plouffe et al. 2012).

PYR, in combination with sulfadoxine, is also used for prophylaxis purposes in vulnerable populations in endemic countries. Per WHO recommendations, pregnant women receive IPTp, the recommended regimen is once a month and the adult dose contains 75 mg of PYR (WHO 2014). In children receiving seasonal malaria chemoprophylaxis with sulfadoxine– PYR plus amodiaquine, the medications are dosed by age (WHO 2013).

In addition, PYR can be used for treatment of toxoplasmosis at higher doses than utilized for malaria treatment or chemoprophylaxis (75-100 mg per day) and given for prolonged periods of time (weeks to months), usually in conjunction with sulfadoxine and folinic acid. With doses of PYR used for the treatment of toxoplasmosis, side effects such as anorexia and vomiting may occur, but can be minimized by giving the medication with meals.

In this study, we plan to use a higher dose of PYR, 75 mg orally for either one or two days each month. One dose is similar to chemoprophylaxis doses used in IPTp. Administration of 75 mg of PYR for two consecutive days only is similar to PYR dosages utilized in toxoplasmosis treatment, but for a significantly shorter period of time.

The current approved package insert for PYR and additional details on safety of PYR are provided in the Investigator's Brochure.

4.3 PfSPZ Challenge

One of the strains manufactured by Sanaria Inc. is PfSPZ Challenge NF54. PfSPZ Challenge contains fully infectious PfSPZ purified from the salivary glands of *Anopheles stephensi* mosquitoes raised under aseptic conditions. The infectious PfSPZ are formulated in cryoprotectant to maintain potency for an extended period. Sanaria® PfSPZ Challenge (NF54) is known to be susceptible to chloroquine, PYR, atovaquone, artesunate, but not mefloquine. More detailed product information can be found in **Sections 7.1** and the Investigator's Brochure.

The clinical studies for PfSPZ Challenge are detailed in **Section 1.4.2**

4.4 Description of Intervention

The intervention in this study involves the injection by DVI of either $4.0x10^5$ or $3.0x10^5$ aseptic, purified, cryopreserved, fully infectious PfSPZ with concurrent administration of antimalarial drugs to attenuate the parasites *in vivo*. The goal is to induce stage specific immunity to Pf malaria (PYR = pre-erythrocytic immunity; chloroquine = pre-erythrocytic immunity + possibly erythrocytic immunity). Following immunization, participants will be followed through the transmission season in order to assess VE. Antimalarial (Coartem®) will be administered to all participants twice during the study, approximately 2 weeks prior to the first vaccination in order to clear pre-existing parasitemia that may interfere with immunity development. The second dose will be given approximately 2 weeks prior to the 3rd vaccination (see **Appendix A**) in order to clear any parasitemia infection that may have happened naturally during the vaccination period and prior to the follow up period.

Pilot Study

- ***Pyrimethamine Pilot (Arms 1a, 2a, 5a and 6a)*:** 1 dose of $4.0x10^5$ PfSPZ Challenge **AND** either 1 dose of 75 mg PYR will be administered on day 0 (*Arm 1a*) ***or*** 2 doses

of 75 mg PYR administered on day 2&3 (*Arm 2a*) post PfSPZ Challenge. Dosing on day 0 will occur within +/- 1 hour of PfSPZ Challenge injection. Subjects will be followed daily with sensitive thick blood smears from day 6 through day 12 post PfSPZ Challenge. Participants will first be treated with full dose of artemether/lumefantrine approximately 2 weeks prior to vaccination (see **Appendix A**). In addition, all participants will be treated with full dose of artemether/lumefantrine at the end of the pilot, starting at day 12 post injection or if diagnosed with clinical malaria per Malian guidelines prior to day 12 days post injection. Samples for assessment of subpatent parasitemia breakthrough by qPCR will be collected from day 6 through day 12 post PfSPZ Challenge injection and will be run retrospectively. See **Section 3.2** for more details. If there is patent parasitemia, *Arm 5a* or *6a* will be enrolled to receive a decreased dose of PfSPZ Challenge, $3.0x10^5$ PfSPZ.

- ***Chloroquine Pilot (Arm 3a)*:** 1 dose of PfSPZ Challenge ($4.0x10^5$ PfSPZ) **AND** chloroquine beginning with a loading dose 2 days prior to PfSPZ Challenge (1000 mg = 600 mg base) and finishing with a maintenance dose 5 days after PfSPZ Challenge (500 mg = 300 mg base) (*Arm 3a*). Subjects will be followed daily with blood smears from day 6 through day 12 post PfSPZ Challenge. All participants will first be treated with full dose of artemether/lumefantrine approximately 2 weeks prior to vaccination (see **Appendix A**). In addition, all participants will be treated with full dose of artemether/lumefantrine at the end of the pilot, starting at day 12 post injection or if diagnosed with clinical malaria per Malian guidelines prior to day 12 days post injection. See **Section 3.2** for more details.

Main Study

- ***Pyrimethamine Main (Arms 1b/5b and 2b/6b)*:** 3 doses at 28 days intervals of $4x10^5$ PfSPZ **AND** *either* 1 dose of 75 mg of PYR given on day 0 (*Arm 1b*) *or* on day 2 & 3 (*Arm 2b*) post vaccination *Arms 5b* or *6b* will be enrolled if necessary as determined by the pilot to receive a decreased dose of PfSPZ Challenge, $3x10^5$ PfSPZ. These two arms will not receive chloroquine. Dosing on each day 0 will occur within +/- 1 hour of PfSPZ Challenge injection. Subjects will be followed clinically. During the immunization phase, samples for blood smears and malaria qPCR will collected from 7 through 9 days post injection and assays will be conducted retrospectively as outlined in **Appendix A**. To guide clinical management, a blood smear will be done and read in real time if a participant presents with symptoms. Approximately one to two weeks prior to the first vaccination and the third vaccination, as outlined in **Appendix A**, all participants will be treated with a full three-day course of artemether/lumefantrine to clear any naturally transmitted parasitemia. All participants will again be treated with standard dose of artemether/lumefantrine at the time of diagnosis of clinical malaria per Malian ministry of health guidelines (a presence of a positive malaria test + clinical symptoms) (see **Section 3.3** for more details). If a participant is diagnosed with clinical malaria and treated with artemether/lumefantrine prior to 3rd vaccination, scheduled dosing of artemether/lumefantrine will not be repeated if the treatment falls within the administration visit windows outlined in **Appendix A.**

- ***Chloroquine Main (Arm 3b):*** 3 doses of PfSPZ Challenge ($4.0x10^5$ PfSPZ) separated by 28-day intervals **AND** chloroquine beginning with a loading dose 2 days prior to PfSPZ Challenge (1000 mg = 600 mg base) and continuing weekly using a maintenance dose (500 mg = 300 mg base) for a total of 10 doses, finishing with a last dose 5 days after the third injection with PfSPZ Challenge. If needed as determined in the pilot phase, NSAIDs may be administered on days 7 & 8 post the first PfSPZ Challenge DVI to diminish symptoms. Subjects will be followed clinically. During the immunization phase, samples for blood smears and malaria qPCR will be collected from 7 through 9 days post injection and assays will be conducted retrospectively as outlined in **Appendix A.** To guide clinical management, a blood smear will be done and read in real-time if a participant presents with symptoms. Approximately one to two weeks prior to the first and the third vaccination as outlined in **Appendix A**, all participants will be treated with a full three-day course of artemether/lumefantrine to clear any naturally transmitted parasitemia. All participants will again be treated with standard dose of artemether/lumefantrine at the time of diagnosis of clinical malaria per Malian ministry of health guidelines (presence of a positive malaria test + clinical symptoms) (see **Section 3.3** for more details). If a participant is diagnosed with clinical malaria and treated with artemether/lumefantrine prior to 3rd vaccination, scheduled dosing of artemether/lumefantrine will not be repeated if the treatment falls within the administration visit windows described in **Appendix A.**

- ***Placebo Controls (Arms 4)***: Participants in this group will receive NS injections following the same schedule as vaccinated arms while on PYR dosed either on day 0 (*Arm 4a*) or day 2 & 3 (*Arm 4b*) post vaccination or weekly chloroquine (*Arm 4c*). These participants will follow the same study procedures (**Appendix A**) as the corresponding vaccinated arm that is receiving the same drug regimen. They will not receive PfSPZ Challenge injections. Approximately one to two weeks prior to the first and the third vaccination as outlined in **Appendix A**, all participants will be treated with a full three-day course of artemether/lumefantrine to clear any naturally transmitted parasitemia. All participants will again be treated with standard dose of artemether/lumefantrine at the time of diagnosis of clinical malaria per Malian ministry of health guidelines (presence of a positive malaria test + clinical symptoms) (see **Section 3.3** for more details).

Vaccine efficacy will be evaluated against naturally transmitted *Plasmodium falciparum* (Pf) malaria infection. All participants enrolled in the main arm will be evaluated for infection by blood smears through the transmission season starting two weeks to approximately 6 months after the third vaccination.

The chemoprophylactic agents used in the current study are PYR and chloroquine. Both PYR and chloroquine are commercially available FDA-approved antimalarial drugs for treatment and prophylaxis of Pf. Chloroquine dosing used in the study is abbreviated from the standard weekly suppressive prophylaxis in non-immune populations (600 mg base loading dose, then 300 mg base weekly maintenance dose for a total of 4 weeks after Pf exposure) given that in this study the day of exposure to SPZ is precisely known, there is no risk of *P. vivax* co-infection, and the biology of the Pf NF54 parasite is well characterized. Thus, the loading dose

will be administered 2 days prior to PfSPZ Challenge and continued weekly until 5 days after the third dose of PfSPZ Challenge (or the fourth dose in the booster phase as outlined below and in **Appendix A**). In prior studies, administering the last dose of chloroquine in this manner has been sufficient to eliminate parasitemia in all study subjects during PfSPZ-CVac (chloroquine) immunization (Mordmuller, Surat et al. 2017**)** and observed in our previous study, NIAID protocol #15-I-0169 and #17-I-0067 as described in **Section 1.4.2.**

PYR dosing in this study will be daily dosing of 75 mg for one or two consecutive days, dosed on day 0 versus days 2 and 3 after either $4x10^5$ PfSPZ or $3x10^5$ PfSPZ of PfSPZ Challenge or NS (**Table 14**). The planned dosing of 75 mg is consistent with the doses used for IPTp or toxoplasmosis treatment. During the main phase, there will be a total of 3 doses (*Arm 1b/ 5b*) or 6 doses (*Arm 2b/6b*) of PYR received during the study.

Booster Phase

- ***Pyrimethamine Booster (Arms 1b and 2b)*:** 1 additional dose (and the fourth cumulative dose) of $4x10^5$ PfSPZ Challenge will be given approximately 11 months after third vaccinations **AND** 1 dose of 75 mg of PYR given on day 0 (*Arm 1b*) *or* on day 2 & 3 (*Arm 2b*) post vaccination. These two arms will not receive chloroquine. Dosing on each day 0 will occur within +/- 1 hour of PfSPZ Challenge injection. Subjects will be followed clinically. During the booster immunization phase, samples for blood smears and malaria qPCR will collected from 7 through 9 days post injection and assays will be conducted retrospectively as outlined in **Appendix A**. To guide clinical management, a blood smear will be done and read in real time if a participant presents with symptoms. Approximately one to two weeks prior to the fourth vaccination, as outlined in **Appendix A**, all participants will be treated with a full three-day course of artemether/lumefantrine to clear any naturally transmitted parasitemia. All participants will again be treated with standard dose of artemether/lumefantrine at the time of diagnosis of clinical malaria per Malian Ministry of Health guidelines (a presence of a positive malaria test + clinical symptoms) (see **Section 3.3** for more details). During the booster phase, there will be a total of 1 dose (*Arm 1b*) or 2 doses (*Arm 2b*) of PYR received.

- ***Chloroquine Main (Arm 3b):*** 1 additional dose (and the fourth cumulative dose) of $4.0x10^5$ PfSPZ Challenge will be given approximately 11 months after third vaccinations **AND** chloroquine beginning with a loading dose 2 days prior to PfSPZ Challenge (1000 mg = 600 mg base) and continuing for one additional week using a maintenance dose (500 mg = 300 mg base) 5 days after the booster injection with PfSPZ Challenge for a total of 2 doses. Subjects will be followed clinically. During the immunization phase, samples for blood smears and malaria qPCR will be collected from 7 through 9 days post injection and assays will be conducted retrospectively as outlined in **Appendix A.** To guide clinical management, a blood smear will be done and read in real-time if a participant presents with symptoms. Approximately one to two weeks prior to the fourth vaccination as outlined in **Appendix A**, all participants will be treated with a full three-day course of artemether/lumefantrine to clear any naturally transmitted parasitemia. All participants will again be treated with standard dose of artemether/lumefantrine at the time of diagnosis of clinical malaria per Malian Ministry of Health guidelines

(presence of a positive malaria test + clinical symptoms) (see **Section 3.3** for more details).

- ***Placebo Controls (Arms 4)***: Participants in this group will receive NS injections following the same schedule as vaccinated arms while on PYR dosed either on day 0 (*Arm 4a*) or day 2 & 3 (*Arm 4b*) post vaccination or weekly chloroquine (*Arm 4c*). These participants will follow the same study procedures (**Appendix A**) as the corresponding vaccinated arm that is receiving the same drug regimen. They will not receive PfSPZ Challenge injections. Approximately one to two weeks prior to the fourth vaccination as outlined in **Appendix A**, all participants will be treated with a full three-day course of artemether/lumefantrine to clear any naturally transmitted parasitemia. All participants will again be treated with standard dose of artemether/lumefantrine at the time of diagnosis of clinical malaria per Malian Ministry of Health guidelines (presence of a positive malaria test + clinical symptoms) (see **Section 3.3** for more details).

Vaccine efficacy will be evaluated against naturally transmitted *Plasmodium falciparum* (Pf) malaria infection. All participants enrolled in the booster phase will be evaluated for infection by blood smears through the transmission season starting two weeks to approximately 6 months after the fourth (booster) vaccination.

Table 14. Chemoprophylaxis regimen and assignment

Intervention	Arm Assignment	Dosing
Antimalarials		
Pyrimethamine	Arm 1 Arm 2 Arm 4a &b Arm 5 Arm 6	Three single strength pills (75 mg PYR total) given on day 0 (*Arms 1, 4a, 5*) or days 2& 3 (*Arms 2, 4b, 6*) following each administration of PfSPZ Challenge by DVI during PfSPZ-CVac immunization. The pilot arms will receive either a total of 1 daily dose (*Arms 1a, 5a) or* 2 daily doses (*Arms 2a, 6a*). The main arms will receive a total of 3 daily doses (*Arm 1b/5b),* or 2 daily doses for a total of 6 daily doses (*Arm 2b/6b*). The booster phase will receive a total of 1 dose (*Arm 1b),* or 2 daily doses (*Arm 2b*).
Chloroquine	Arm 3 Arm 4c	Loading dose of approximately 1000 mg chloroquine phosphate (600 mg chloroquine base) 2 days prior to first administration of PfSPZ Challenge by DVI during PfSPZ-CVac immunization. Then 500 mg (300 mg base) 5 days after 1st injection (*Arm 3a,* 2 doses total) OR weekly until 5 days after 3rd injection (*Arms 3b* and *4c* 10 doses total). The booster phase will receive an additional loading dose 2 days prior to 4th injection of PfSPZ Challenge (1000 mg = 600 mg base) and one additional maintenance dose (500 mg = 300 mg base) 5 days after the booster injection for a total of 2 additional doses.
Artemether/ lumefantrine	ALL Arms	A total of 6 doses administered twice daily of single tablet of artemether 80 mg/lumefantrine 480 mg (Full course) approximately 14 days prior to first vaccination and end of study participation in the pilot phase (*Arms 1a, 2a, 3a, 5a, 6a*). In the main phase, full course will be administered approximately 7-14 days prior to the 1st and 3rd injection (*Arms 1b/5b, 2b/6b, 3b, 4*). In the booster phase, full course will be administered approximately 7-14 days prior to the 4th injection (*Arms 1b, 2b, 3b, 4*). A full course will also be administered at the time of clinical malaria diagnosis (ALL Arms)
P. falciparum sporozoites (PfSPZ), non-attenuated		
PfSPZ Challenge (NF54) administered by DVI for **PfSPZ-CVac** immunization	Arm 1 Arm 2 Arm 3 Arm 5 Arm 6	**$4.0x10^5$ (*Arms 1, 2 and 3*) or $3.0x10^5$ (*Arms 5 and 6*)** aseptic, purified, cryopreserved, non-attenuated PfSPZ of PfSPZ Challenge (NF54) administered by DVI.
Placebo Control		

Normal saline administered by DVI	Arm 4	Sterile normal saline will be administered by DVI
Note: All medication administration timing will adhere to visit windows outlined in **Appendix AAppendix A**		

4.5 Presumptive Antimalarial Treatment with Artemether/Lumefantrine

All participants will undergo parasitemia clearance with full course of artemether/lumefantrine treatment twice during the course of the study, approximately one to two weeks prior to first and the third vaccinations and approximately two weeks prior to fourth (booster) vaccinations.
Note: In *Arms 3a, 3b* and *4c*, first dose of AL (Study Day -17) should be administered 14 days or more prior to CQ loading dose (Study Day -2)
During the Pilot Study, participants will be treated at 12 days after PfSPZ Challenge injection under direct observation by study staff.

In addition, during vaccination and follow up period, participants diagnosed with clinical malaria in accordance with the Malian Government treatment guidelines, will be treated with standard regimen of artemether/lumefantrine. Treated participants will continue to be monitored closely until any residual symptoms of malaria are mild or resolved.

5 Study Endpoints

5.1 Primary Endpoints

Pilot Phase

- Incidence of positive sBS occurring after PfSPZ-CVac immunization starting on day 7 post DVI (*Arms 1a, 2a, 5a, 6a*).
- Incidence and severity of local and systemic grade 3 signs or symptoms lasting more than 48 hours despite adequate management and SAEs occurring after PfSPZ-CVac DVI (*Arm 3a)*

Main Phase and Booster Phase

- Incidence and severity of local and systemic adverse events (AEs) and SAEs occurring after PfSPZ-CVac immunization. (*Arms 1b/5b, 2b/6b, 4a, 4b)*

- Incidence of clinical malaria diagnosis occurring after PfSPZ-CVac immunization as defined by the occurrence of grade 3 signs or symptoms lasting more than 48 hours despite adequate management. *(Arm 3b, 4c)*

5.2 Secondary Endpoints

Protective Efficacy

***P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood starting 2 weeks after the 3rd vaccination in the Main Phase *(Arm 1b/5b, 2b/6b, 3b, 4)* and then 2 weeks after the 4th vaccination for approximately 6 months for the Booster Phase.**

5.3 Exploratory Endpoints

Pyrimethamine regimen efficacy and Immunogenicity

- *P. falciparum* blood stage infection defined as detection of *P. falciparum* parasites by qPCR following PfSPZ Challenge. (*Arm 1, 2, 5, 6)*
  - Humoral immune responses after PfSPZ-CVac regimens by assessing antibodies to PfSPZ, Pf asexual erythrocytic stages (AES), and specific Pf sporozoite, liver and blood-stage antigens such as CSP, MSP-1, AMA-1 in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups. *(All Arms)*
  - Cellular immune responses after PfSPZ-CVac regimens to PfSPZ, PfAES, and specific Pf sporozoite, liver and blood-stage antigens, such as CSP, MSP-1, AMA-1, in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups. *(All Arms)*
  - Cellular and humoral responses that correlate with protective efficacy
  - Prolonged prepatent period and/or reduced density and duration of parasitemia in those developing parasitemia after CHMI *(Arms 1, 2, 3, 5, 6)*
  - Comparison of γδ T cells before and after PfSPZ-CVac immunization and malaria infection during transmission season using *ex vivo* whole blood or *in vitro* staining. *(All Arms)*

5.4 Sample Size and Estimated Duration of Study

A total of up to 440 participants are expected to be enrolled in this trial (**8**, **9**). Participants in the pilot arms will be monitored actively for about 1 month and those in the main arms will be monitored actively for about 10 months. Up to 800 subjects will be screened to accommodate possible screen failures.

6 Study Population

6.1 Description of Population and Site

Bancoumana is located 60 kilometers southwest of Bamako and has a population of about 9,000 people. The site is situated in the south-Sudanian area of Mali. The climate is hot, with daily temperatures ranging from 19°C to 40°C. The annual rainfall varies between 600 mm and 1200 mm and occurs from June to October. Many clinical trials, as well as epidemiological and entomologic malaria studies, have been done in Bancoumana. Healthy adult subjects will be

recruited from the surrounding community and villages, and the demographics of the study population will therefore be representative of that community.

6.2 Recruitment

Community permission will be obtained from village elders and other community members in Bancoumana after explanation and discussion of the study at a community meeting (see **Section 17.2.1.1**). A general announcement inviting household and family members to the participating clinic to learn about the study will be made at the time of community permission, using local radio or any traditional channel of communication.

6.3 Inclusion Criteria

All of the following criteria must be fulfilled for a subject to participate in this trial:

1. Age ≥ 18 and ≤ 50 years (for booster phase, age ≥ 18 and ≤ 52 years)
2. Resident of Bancoumana or nearby areas
3. In good general health and without clinically significant medical history
4. Malaria comprehension exam completed, passed (a score of ≥80% or per investigator's discretion) and reviewed prior to enrollment (not required for booster phase)
5. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process
6. Willing to have blood samples stored for future research
7. Available for the duration of the study
8. Females of childbearing potential must be willing to use reliable contraception (as defined below) from 21 days prior to first PfSPZ Challenge injection to 28 days following last PfSPZ Challenge exposure (or equivalent study day for *Arm 5* controls). For the booster phase, this applies from 21 days prior to the booster vaccination to 28 days post booster vaccination.
    - Reliable methods of birth control include **one** of the following: confirmed pharmacologic contraceptives (parenteral) delivery; intrauterine or implantable device. OR
    - Reliable methods of birth control include concurrent use of a pharmacologic and a barrier method, i.e., **two** of the following: confirmed pharmacologic contraceptives (oral, transdermal) delivery or vaginal ring **AND** condoms with spermicide or diaphragm with spermicide. OR
    - Non-childbearing women will also be required to report date of last menstrual period, history of surgical sterility (i.e. tubal ligation, hysterectomy) or premature ovarian insufficiency (POI), and will have urine or serum pregnancy test performed per protocol.
9. For booster phase only: previously or currently enrolled in protocol #19-I-N099 and completed all three primary vaccinations.

6.4 Exclusion Criteria

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

1. Pregnancy, as determined by a positive urine or serum human choriogonadotropin (β-hCG) test (if female)
    a. NOTE: Pregnancy is also a criterion for discontinuation of any further dosing or non-safety related interventions for that subject.
2. Currently breast-feeding (if female)
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the participant to understand and comply with the study protocol
4. Hemoglobin, WBC, absolute neutrophils, and platelets outside the local laboratory-defined limits of normal (subjects may be included at the investigator's discretion for 'not clinically significant' values)
5. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal (subjects may be included at the investigator's discretion for 'not clinically significant' values)
6. Infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B (HBV) (For the booster phase: re-testing NOT required for enrollment unless clinically indicated)
7. Known or documented sickle cell disease by history (Note: known sickle cell trait is NOT exclusionary)
8. Clinically significant abnormal electrocardiogram (ECG) (For the booster phase: re-testing NOT required for enrollment unless clinically indicated)
9. Moderate or high risk for coronary heart disease (CHD) based on NHANES I cardiovascular risk assessment (**Appendix C**)
10. 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
11. History of receiving any other investigational product within the past 30 days
12. Participation or planned participation in a clinical trial with an investigational product prior to completion of the last required protocol follow-up visit
13. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
14. History of a severe allergic reaction or anaphylaxis
15. Severe asthma (defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years, or that has required the use of oral or parenteral corticosteroids at any time during the past 2 years)
16. 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
17. Known immunodeficiency syndrome

18. Known asplenia or functional asplenia
19. Use of:
    a. Chronic (≥14 days) oral or IV corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of vaccination
    b. **Antimalarials** for example artemether, artemether-lumefantrine, artesunate, artesunate-amodiaquine, other than those prescribed by the investigator as part of the study procedures within 14 days prior to the first vaccine
    c. **Systemic antibiotics** with known antimalarial activity such as trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, ciprofloxacin or azithromycin within 5 half-lives of the drug prior to the first vaccine
20. Receipt of a live vaccine within the past 4 weeks or a killed vaccine within the past 2 weeks prior to Vaccination #1 and every subsequent vaccination day
21. Receipt of immunoglobulins and/or blood products within the past 6 months
22. Previous receipt of an investigational anti-infectivity malaria vaccine in the last 2 years (this requirement is waived for the booster phase)
23. Known allergies or contraindication against: PYR, chloroquine, NSAIDs, artemether, lumefantrine
24. 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 Study Agents

7.1 PfSPZ Challenge (NF54)

Sanaria® PfSPZ Challenge consists of aseptic, purified, cryopreserved PfSPZ produced by the biotechnology company Sanaria Inc. (Rockville, MD, USA) (Hoffman, Billingsley et al. 2010, Epstein, Tewari et al. 2011, Roestenberg, Bijker et al. 2013). In brief, manufacture includes the production, under traditional environmental conditions, of eggs from a colony of *A. stephensi* mosquitoes housed in a controlled environmental chamber. Surface disinfection of the eggs is performed by exposure to chemical agents in a Class II biosafety cabinet. From this point forward, all materials and products are handled using aseptic methods to ensure that contaminating microorganisms are not introduced to and carried through the process. Surface-disinfected eggs are inoculated into sterile, vented flasks containing aseptic growth medium. The eggs hatch and develop into pupae, which are transferred to an adult mosquito container where the adult mosquitoes emerge. These adult mosquitoes, which have been raised under aseptic conditions, are fed Pf gametocyte-infected blood in an aseptic, High-Security Insectary. The *P. falciparum* gametocyte-infected blood produced from cultures of the Pf isolate NF54 (Pf NF54) derived from a working cell bank (WCB) of the well- characterized Pf NF54 isolate. Infected adult mosquitoes are maintained under aseptic conditions. Pf SPZ migrate to the salivary glands. The salivary glands from the PfSPZ infected mosquitoes are removed by hand dissection under aseptic conditions. PfSPZ are then purified from the salivary glands, counted, and, at a specified concentration, cryopreserved. Cryopreservation

commences with the addition of cryoprotective additives to produce the PfSPZ Challenge product. PfSPZ Challenge is dispensed into screw-cap vials and stored in liquid nitrogen vapor phase (LNVP) at 150°C to -196°C. All the procedures are described in more detail in the investigator's brochure.

7.2 Phosphate buffered saline and human serum albumin diluent

The diluent for PfSPZ Challenge is composed of phosphate-buffered saline (PBS) and human serum albumin (HSA). Vials of PBS and HSA will be shipped or transported to the clinical site, where diluent composed of PBS and HSA is prepared according to standard operating procedures (SOPs) provided by Sanaria. PBS that will be used will be released with a Certificate of Analysis (CoA) supplied by Sanaria. The PBS is stored at room temperature (15°C to 30°C) in a controlled room.

HSA is a licensed product, which is approved for parenteral, IV administration to humans and is purchased by Sanaria from CSL Behring (Kankakee, IL, USA). Every lot of HSA purchased is supplied with a CoA that is reviewed and approved upon receipt at Sanaria. The HSA lots are extensively tested to ensure that it is free of infectious agents as listed in the CoA and is approved for use by the FDA, US license number 1766. HSA vials are stored at room temperature (15°C to 30°C) in a controlled room.

7.3 Storage and Handling of PfSPZ Challenge

Shipment of PfSPZ Challenge in LNVP is in compliance with all FDA, U.S. Department of Transportation, and United Nations transport guidelines for shipping bio-hazardous materials on dry ice and LNVP. Transfer, receipt and maintenance of PfSPZ Challenge from its storage site to the clinical trial site will follow SOPs provided by Sanaria. During transport and at the study site, the LNVP shipper will be monitored. Receipt of the PfSPZ Challenge will be documented on a tracking log by study staff.

7.4 Control Product

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

7.4.1 Storage and Handling

The NS is stored at room temperature in a controlled room per product standards. Each NS 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).

7.4.2 Disposition and Dispensation

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

7.4.3 Administration and Dosage

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

7.4.4 Accountability

NS 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.

7.5 Pyrimethamine, Chloroquine, Artemether/Lumefantrine

Chloroquine, Pyrimethamine, and Artemether/Lumefantrine are commercially available antimalarial drugs. Package inserts for all three drugs are provided.

CQ is supplied and manufactured in tablets of 250 mg or 500 mg (equivalent to 150 or 300 mg CQ base). Based on availability, CQ from the U.S, Europe or Asia may be obtained under approval from the Mali Ministry of Health.

PYR is manufactured in tablets of 25 mg. Based on availability, PYR from the U.S, Europe or Asia may be obtained under approval from the Mali Ministry of Health.

Artemether/Lumefantrine is manufactured in tablets of 20 mg artemether and 120 mg lumefantrine. Artemether/Lumefantrine is readily available for standard treatment of malaria in Mali thus it will be obtained in Mali.

7.5.1 Pyrimethamine, Artemether/Lumefantrine and Chloroquine Packaging and Labeling

PYR and chloroquine tablets will be maintained in the manufacturer's original packaging until prepared for dispensing in the trial.

Artemether/Lumefantrine though not drugs under study but drugs that all participants will receive, will be maintained in the same manner as PYR and chloroquine.

7.5.2 Pyrimethamine, Chloroquine, Artemether/Lumefantrine Accountability

7.5.2.1 Receipt

PYR, chloroquine, and artemether/lumefantrine, tablets will be purchased from reliable, commercial sources and provided to the local pharmacist and clinical team. Drug accountability will be managed as outlined in the Pharmacy Manual.

7.5.2.2 Preparation and Administration

PYR, chloroquine, and artemether/lumefantrine will be provided as tablets for oral administration with food. Administration is under direct observation by study staff according to dosing parameters. Individual doses of PYR,

chloroquine, and artemether/lumefantrine will be prepared by study staff as described in the Pharmacy Manual.
Note: The evening doses of artemether/lumefantrine will be given to participants to take at home therefore will not be under direct observation. Exceptions to direct observation for artemether/lumefantrine may be made at the discretion of the PI for extenuating circumstances, such as to accommodate religious festivals.

7.5.2.3 Storage and Handling

PYR, chloroquine, and artemether/lumefantrine tablets will be maintained in the manufacturer's original packaging and stored at the clinic under recommended storage conditions until prepared for dispensing.

7.5.2.4 Return of Study Product

Final accountability of drug supplies will be performed at the conclusion of the study. Final disposition of any remaining PYR, chloroquine, and artemether/lumefantrine will be determined and documented. PfSPZ Challenge, HSA and PBS inventory will be tracked and recorded and returned to Sanaria for final disposition and accountability according to Sanaria SOP.

8 STUDY 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.

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

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

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

- Explain the study and informed consent document to the subject. For the booster phase, informed consent can be signed within 90 days prior to the 4th vaccination.
- Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.

- Ensure the subject has correctly answered ≥80% of the questions (see **Section 17.2.1.2**) on the Malaria Comprehension Exam.
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females, and medication use.
- Confirm that females of childbearing potential are willing to use reliable contraception from 21 days prior to first PfSPZ Challenge injection through 28 days after the third injection in the main phase. For the booster phase, willingness to use reliable contraception should be confirmed from at least 21 days prior to the fourth vaccination through 28 days after the fourth vaccination.
- Administer a complete physical examination, including vital signs (height, weight, blood pressure, temperature, and heart rate).
- Complete HIV pre- and post-test counseling as indicated, including follow-up contact with subject to report the results and referral for appropriate medical care if indicated.
- Obtain approximately 10 mL of blood for complete blood count (CBC) with differential and platelet count, ALT, Cr, hepatitis B testing, hepatitis C testing, HIV testing. For the booster phase: HIV, hepatitis B, and hepatitis C testing will only be repeated if clinically indicated.
- Obtain urine (or serum) for pregnancy testing (for females) and urinalysis/urine dipstick for protein and blood.
- Obtain a 12-lead ECG. For booster screening, an ECG will only be repeated if clinically indicated.

Final eligibility will be determined after all required information is obtained. 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.

If the screening visit was completed outside of the 56 days to first vaccination window, except for hepatitis B, hepatitis C and HIV testing, **ALL** remaining screening procedures listed above, including consent and comprehension exam, will need to be repeated and documented.

8.2 Assignment of Groups

Enrollment in the main phase occurs with receipt of the first dose of artemether/lumefantrine and collection of baseline study samples on study Day -17..

In the pilot *Arms*, participants will be randomized into *Arm 1a or 2a.* Randomization will occur prior to enrollment, prior to or on study Day -17. If a subject was randomized into any arm but was not vaccinated, they may be randomized or enrolled in a subsequent *Arm*. If enrollment into *Arm 5a or 6a* is necessary, these participants will be enrolled sequentially. **Note:** If enrollment into *Arms 5a* and *6a* occurs prior to completion of *Arms 1a, 2a* or *3a*, the participants will be randomized into either *Arm 5a* or *6a*.

For the main *Arms*, participants in *Arms 1b/5b*, *2b/6b, 4a, and 4b* (first year) *AND 3b, and 4c* (subsequent year) will be randomized. Randomization will occur prior to enrollment, prior to or on study Day -17
If a subject withdraws from the study after randomization, they can be replaced by another subject at random if withdrawal happens prior to receipt of first vaccination. Once a subject has received their first vaccination, they cannot be replaced. **Note:** A replacement subject may receive artemether/lumefantrine within 14 days of receiving first vaccination.
On vaccination days, the vaccines associated with each randomization number will be obtained from the pharmacist.

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

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. Fingerprinting may also be implemented to ensure subjects are identified correctly during the visits. The biometric application that would be used is the Biometric Screening Log application developed by the data management unit of University Clinical Research Center (UCRC) of the University of Science, Techniques and Technology of Bamako (USTTB) in Mali. This system utilizes FBI-certified fingerprint scanners to scan participant fingerprints. In addition to providing identity verification during scheduled and unscheduled visits to the site at key stations (Identification Point, Clinic Exam Room, Lab/Phlebotomy, and Vaccination rooms), the software provides the following functionality:

- ID card printing with QR code on a Zebra ID card printer
- Participant-specific labels with 2 or 3 dimensional barcodes for samples, also printed from the Zebra printer
- Dynamic visit calendar for full week
- Daily report generated on visits that are completed, pending, missed, and scheduled
- Weekly summary report available on study dashboard

When the biometric system is implemented, participants' fingerprints may be collected and stored. Neurotechnology's VeriFinger SDK fingerprint compression algorithm will be used to create a secure template. This algorithm meets accuracy requirements outlined in the Wavelet Scalar Quantization (WSQ) Gray-Scale Fingerprint Image Compression Specification developed by the FBI and NIST. PII and biometric templates are saved on a SGDBR SQL Server database compliant with Title 21 of the Code of Federal Regulations. The HF SQL database is installed on a local server. The server is located in a secure area with limited access (physical and logical access security, temperature monitoring, fire and smoke detection, camera surveillance). Regular backup will be done based on data changes that will be encrypted and stored on local servers to prevent any leak of PII to NIH servers and or to NIH personnel.

8.3 **Detailed Study Procedures**

Detailed study procedures are outlined in **Appendix A**.

8.4 **Indications for Deferral of Sanaria® PfSPZ Challenge**

If any one of the following AEs occurs at the time of the scheduled Sanaria® PfSPZ Challenge, the subject may either receive the Sanaria® PfSPZ Challenge at a later date within the allowable time interval specified in the protocol or withdrawn at the discretion of the Investigator:

- Oral temperature >38.0˚C at the time of vaccination will warrant deferral of injection until fever resolves (within protocol-defined vaccination window).

- Any other condition that in the opinion of the Investigator poses a threat to the individual if injection or that may complicate interpretation of the outcome variables following injection.

Such individual(s) will be followed in the clinic until the symptoms resolve or the window for Sanaria® PfSPZ Challenge expires.

9 Study Procedures

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 (Hb), and platelet count.
2. Serum creatinine (Cr)
3. Alanine aminotransferase (ALT)
4. Hepatitis B surface antigen (HBsAg) test (can include rapid diagnostics, enzyme-linked immunosorbent assay [ELISA], PCR if indicated) (at screening only in the main phase. For the booster phase, ONLY if clinically indicated)
5. HCV test (can include rapid diagnostics, ELISA, PCR if indicated) (at screening only in the main phase. For the booster phase, ONLY if clinically indicated)

6. HIV test (can include rapid diagnostics, ELISA, Western Blot if indicated) (at screening only in the main phase. For the booster phase, ONLY if clinically indicated)
7. Urine dipstick and/or Urinalysis (at screening only)
8. Urine and/or serum pregnancy testing (β-hCG)
9. Hemoglobinopathy testing (for enrolled participants only) -- testing for HbAA, HbAC, HbCC, HbAS, HbSS

9.3 Electrocardiogram

Electrocardiograms (12-lead ECGs) will be performed during screening for the main phase and as needed throughout the study by the study site team in Mali and read by trained study investigators or a cardiologist. Subjects with clinically significant abnormalities will be excluded from the study. Subjects with QTc > 450 ms will be excluded.

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 films will be prepared at the specified time points or when clinically indicated and will be examined by technicians with a documented experience in slide reading following the SOP (ML-005-05) established for malaria diagnosis in Sanaria PfSPZ studies slide reading within the MRTC (Guindo, Shott et al. 2012), and in consideration of the modifications suggested for CHMI trials (Laurens, Duncan et al. 2012). During the pilot study, ALL blood smears, regardless of the presence or absence of symptoms, will be read per procedures followed when evaluating symptomatic participants, depicted as sBS. Slides are considered positive if at least two unambiguous parasites per slide are identified and confirmed by a second microscopist. Positive results will be reported promptly to the study Principal Investigator (PI) or designee.

Thick blood smears will be prepared from the blood remaining in the collection device, or (at time points when no 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 CHMI 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 (Walther, Dunachie et al. 2005, Walther, Thompson et al. 2006). These research molecular assays have significantly increased sensitivity for detection of Pf blood-stage infection approaching 20 parasites/mL, often resulting in diagnosis 2-4 days earlier than by paired thick blood smears (Hermsen, Telgt et al. 2001, Schneider, Schoone et al. 2004, Andrews, Andersen 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 Pf with a detection limit of at least 20 parasites/mL that will be used during the study for comparison to traditional thick blood smears.

Pf qPCR will be performed retrospectively as outlined in the study procedures (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 nucleic acids preservation.

9.5 Unscheduled Blood Smear Positive Visits

Subjects with detection of Pf malaria parasites on thick blood smear after the third vaccination and after the fourth vaccination (booster dose) only (whether or not they were symptomatic; scheduled or unscheduled visits) will be asked to the clinic within 1 calendar day to provide an additional blood sample for the following as seen in **Appendix A**:

- 0.5 mL EDTA microtainer: for whole blood ex-vivo assays if sample not obtained within the last 7 days.
- 4 mL EDTA tube: to obtain RNA and DNA for the study of host and parasite transcriptome (RNA) and parasite genotype (DNA) and to obtain plasma for proteomics studies if sample not obtained within the last 28 days in the main study.

As indicated in Appendix A, a malaria qPCR will be collected at any unscheduled visit at which a blood smear sample is also collected.

10 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 Challenge during vaccination and post CHMI will be performed at the Laboratory of Malaria Immunology and Vaccinology, NIAID, NIH, Sanaria Inc, and NIH Center for Human Immunology, Autoimmunity, and Inflammation (CHI) according to standard laboratory procedures.

These assays include:

1. Binding ELISA for antibodies to circumsporozoite protein (PfCSP), and potentially to other Pf SPZ, liver stage and blood stage antigens (which includes but not limited to: sporozoite surface protein 2, liver stage antigen 1, erythrocyte binding antigen 175 (EBA-175), merozoite surface protein 1, merozoite surface protein 5, and exported protein 1 [PfLSA-1, PfEBA-175 , PfMSP-1, PfMSP-5, EXP-1]

2. (IFN-γ) Enzyme-linked immunosorbent spot assay (ELISPOT) and multi-parameter flow cytometry with intracellular cytokine staining (ICS) 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 and to AES parasites by IFA as described (Epstein, Tewari et al. 2011, Seder, Chang et al. 2013) and also by protein microarrays (Antigen Discovery Inc).

Laboratory assays to assess immune responses to novel pre-erythrocytic antigens may be performed in the PEVA Consortium laboratories at the Laboratory of Malaria Immunology and Vaccinology, NIAID, 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 may 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 PfSPZ-CVac (pyrimethamine) develop immune responses to pre-erythrocytic antigens, and can provide additional data by which to assess the potential for these antigens to be developed as subunit vaccines to prevent infection. The long-term objective of the PEVA consortium is to identify antigens that individually, or in combination with CSP or other antigens, will induce a high level of pre-erythrocytic immunity that is protective against *P. falciparum*.

The assays to be performed include:

1. Binding ELISA for antibodies to *P. falciparum* pre-erythrocytic antigens (PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113)
2. Cellular responses, such as assays of proliferation or cytokine responses (eg, IFN-γ ELISPOT/ICS assay) on peripheral blood mononuclear cells stimulated with *P. falciparum* pre-erythrocytic antigens (PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113)

11 Research Use, Storage, and Tracking of Specimens and Data

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

Storage: Access to stored research samples will be limited using either a locked room or a locked freezer. Temporary storage of samples collected in Mali, prior to shipment to LMIV, may occur at the Core Immunology Laboratory or the MRTC CAP laboratory. Samples will be stored at the LMIV in Rockville, MD/Bethesda, 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; the key to the code will remain with the

MRTC in Mali. 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 use these samples and/or data for research purposes. If the planned research falls within the category of "human subjects research" on the part of the NIH researchers, IRB review and approval will be obtained. This includes the NIH researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.
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 NIH IRB.

Consent to allow long term storage of study samples is a part of the inclusion criteria for this study. However, if a subject decides following enrollment not to have their samples stored, the PI or designee will destroy all known remaining samples and report this destruction to the subject and the 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.

12 Retention of Specimens for Future Use

Specimens collected as part of this trial will be stored for future research. These samples may be used to learn more about malaria infection and other diseases. These samples will not be sold or used to make commercial products. The subject may withdraw permission for future use of specimens at any time. If a subject withdraws his or her permission for future use of specimens, those specimens will be destroyed. All samples stored will be labeled with the subject's study identification (ID) number, which cannot identify the study subject but is linkable to other research databases (e.g., questionnaires, clinical assessments, logbooks) generated by the main study. The database will contain only the study subject's ID number. A master log linking the study subject ID number to the name of the subject will be maintained in a password protected database system with access limited to authorized research team members**.** In the event of samples being requested in the future, only the site Investigators or site study coordinator will have access to the log linking the study subject to the samples.

At the completion of the protocol (termination), samples and data will either be destroyed, stored, or transferred to another existing protocol such as "Research Use of Human Specimens", NIAID Protocol #08-I-N064. In the future, other investigators may wish to study these samples and/or data. If so, the NIH may send samples to the other investigators. If the planned research falls within the category of "human subjects research" on the part of the NIH

researchers, IRB review and approval will be obtained. This includes the NIH researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.

13 Data Sharing Plan

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

14 Assessment of Safety

14.1 Documenting, Recording, and Reporting Adverse Events

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

- immediately documented in the subject's medical record/source document,
- recorded on the electronic database, and
- reported as outlined below (e.g., Investigational New Drug 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 on a study clinician to report an AE, it will be discussed with the PI and documented, recorded, and reported appropriately.

All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in the database. The start date, the stop date, the severity of each reportable event, and the PI's or designee's judgment of the AEs relationship and expectedness to the study agent/intervention will also

be recorded in database.

14.2 **Definitions for the Sponsor**

Adverse Event (AE)
Any untoward medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

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

Suspected Adverse Reaction (SAR)
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 Serious Adverse Event (SAE) is defined as any adverse event that

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. *(examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse)*

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

Unexpected Adverse Event
An AE is considered unexpected if it is not listed in the Investigator's Brochure 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 Suspected Adverse Reaction that is both serious and unexpected.

Protocol Deviation

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

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

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

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

Unanticipated Problem (UP): Any event, incident, experience, or outcome that meets all three of the following criteria would be considered a UP:

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; Investigator's Brochure or other study documents; and

    b. the characteristics of the subject population being studied
2. related or possibly related to participation in the research; and
3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem 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. These events may involve a greater risk of social or economic harm to subjects or others rather than physical/psychological harm. Such events would be considered a non-serious UP. Examples of an UPnonAE include a breach of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

New Onset of Chronic Illness (NOCI)
The new onset of chronic illness 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.

14.3 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 (except for malaria, when in addition each laboratory value will be separately recorded) will be recorded as the AE. Assessment of safety will include clinical observation and monitoring of hematological, chemical, and immunologic parameters. Subjects will be closely monitored for at least 30 minutes following the first dose of any medication and after each administration of PfSPZ Challenge. Additionally, subjects

will return to the clinic for clinical assessments, almost daily during the peak parasitemia period from day 7 through 14 post vaccination in the pilot *Arms*. In the main arms, after first vaccination, participants will be followed approximately every other day on days 7 through 14. After subsequent vaccinations, participants will be seen on every other day on days 7 through 9 post vaccination (**Appendix A**).

The type of information to be collected includes solicited symptoms (direct questioning about known possible side effects of the product), unsolicited symptoms (open-ended questioning such as "do you have any other symptoms"), signs (fever, tenderness, etc.), start date, severity and duration of symptoms, laboratory abnormalities. There will also be an assessment of the degree of relatedness of AEs to investigational product or other study procedures. The information to be captured is summarized below:

Local reactogenicity related to **PfSPZ Challenge** injection – solicited questions include pain, tenderness, redness, swelling, induration, bruising, and pruritus at the injection site:

- Collected for 7 days post administration of PfSPZ Challenge
- Collected from the subject via solicited questions at each study visit

Systemic reactogenicity related to **PfSPZ Challenge** injection – solicited questions include rash, urticaria, pruritus, edema, headache, fever, chills, malaise, myalgia, arthralgia, palpitations, shortness of breath, dizziness/vertigo and non-musculoskeletal chest pain:

- Collected for 7 days post administration of PfSPZ Challenge
- Collected from the subject via symptom solicited questions at each study visit

Systemic symptoms related to **parasitemia/malaria infection** – solicited questions include headache, fever, chills, rigors, sweats, malaise, dizziness, palpitations, shortness of breath, myalgia, arthralgia, nausea, vomiting, abdominal pain, diarrhea, back pain, non-musculoskeletal chest pain:

- Collected from 7-14 days post administration of PfSPZ Challenge during the immunization phase via solicited questions at study visits

Systemic symptoms related to **chloroquine** – solicited questions include headache, nausea, vomiting, diarrhea, abdominal pain, anorexia, dizziness/vertigo tinnitus, change in vision, sleep disturbance, pruritus, rash due to photosensitivity, rash, peripheral neuropathy, anxiety, muscle weakness, weight loss

- Collected from initial loading dose of chloroquine until 6 days post last chloroquine administration
- Collected from the subject via symptom solicited questions at each study visit

Systemic symptoms related to **PYR** – solicited questions include nausea, vomiting, diarrhea, abdominal pain, anorexia, rash

- Collected for 6 days post PYR administration
- Collected from the subject via symptom solicited questions at each study visit

Unsolicited AEs will be assessed throughout the study at each study visit (telephone and clinic follow up) until the final study visit. After that period only unsolicited AEs, SAEs, UPs, and NOCIs will be recorded.

All AEs will be graded for severity and assessed for relationship to the study product. Reactions will be graded as described in this protocol. A study clinician will be available and on call 24 hours a day during the study period. Subjects will be provided contact information and contact cards once enrolled with 24 hour access numbers to the study team. Should a subject call a study clinician to report an AE, it will be fully documented in the subject's case report form (CRF), study chart, and discussed with the PI.

All local and systemic reactions will be captured on the appropriate source documents and database. Those assessed as serious will be further reported on the Sponsor's SAE/UP report form. AEs judged to be possibly, probably, or definitely related to the study product will be followed to adequate resolution. All concomitant medications will be collected throughout the study.

14.3.1 Adverse Event Definitions

A solicited adverse event is defined as a predefined event that is an expected event related to the investigational intervention. These include reactogenicity events following administration of PfSPZ Challenge and events that can be reasonably expected as part of this study as defined in prior similar studies (Chulay, Schneider et al. 1986, Church, Le et al. 1997, Epstein, Rao et al. 2007, Roestenberg, McCall et al. 2009), including local and systemic signs and symptoms related to the PfSPZ Challenge and/or diagnosis of malaria or antimalarial chemoprophylaxis.

Solicited AEs will be captured by direct questioning during the study. Solicited AEs to be recorded as endpoints for this study are provided in **Table 15**. Solicited AEs that are possibly related to chloroquine and/or PYR will be collected for 6 days following each drug administration. Solicited AEs that are possibly related to PfSPZ Challenge injection will be captured at each visit for 7 days (local and systemic symptoms). Symptoms related to malaria secondary to PfSPZ Challenge will be solicited at each visit from 7 to 14 days (systemic symptoms) following each injection of PfSPZ Challenge during the PfSPZ-CVac immunization phase and booster phase.

Additionally, the following rare side effects have been reported with both chloroquine and PYR; these will not be solicited AEs, but relationship will be determined on a subject by subject basis.

Chloroquine: severe changes in mood, psychosis, electrocardiographic change, and hypotension.

PYR: severe hypersensitivity reactions (such as Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme, anaphylaxis), pulmonary eosinophilia, megaloblastic anemia, leukopenia, thrombocytopenia, pancytopenia, atrophic glossitis, cardiac rhythm disorders.

Table 15. Solicited Adverse Events

Solicited Adverse Events

PfSPZ Challenge (local)		
Injection pain/tenderness	Injection swelling/edema	Injection pruritus
Injection erythema/redness	Injection induration	Bruising
PfSPZ Challenge (systemic)		
Rash	Headache	Myalgia
Urticaria	Fever	Arthralgias
Generalized pruritus	Chills/Rigors/Sweats	Dizziness/ Vertigo
Generalized edema	Malaise/Fatigue	Chest Pain (non-skeletal)
Palpitations	Shortness of Breath	
PfSPZ Challenge (malaria)		
Headache	Malaise/ Fatigue	Myalgia
Fever	Nausea	Arthralgias
Chills/Rigors/Sweats	Vomiting	Back Pain
Chest Pain (non-skeletal)	Abdominal Pain	Diarrhea
Shortness of Breath	Palpitations	Dizziness/Vertigo
Chloroquine phosphate		
Anorexia	Diarrhea	Sleep Disturbance
Nausea	Dizziness/Vertigo	Pruritus
Vomiting	Tinnitus	Photosensitivity
Abdominal Pain	Change in Vision	Peripheral Neuropathy
Headache	Malaise/Fatigue	Muscle Ache or Weakness
Anxiety	Weight Loss	Rash
Pyrimethamine		
Nausea	Diarrhea	Anorexia
Vomiting	Abdominal Pain	Rash
Laboratory Results		
ALT (increased ALT)	Hgb (decreased Hgb)	WBC (increased WBC, decreased WBC)
Cr (increased Cr)	Platelets (decreased platelets, increased platelets)	ANC (decreased neutrophil count)
		ALC (increased lymphocytes, decreased lymphocytes)

14.3.2 Severity

Severity of AEs will be assessed by the investigator in
Appendix B. AEs not included in the
Appendix B will be graded for severity using the followings definitions as seen in **Table 16**.

Table 16. Definitions for Severity of AE Grading

Severity	Definition
Grade 1 (Mild)	No interference with activity, may use 1 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

14.3.3 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
- Whether or not there is another identifiable cause

All local (injection-site) reactions will be considered causally related to vaccination.
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 as **related** (including possibly, probably, or definitely) or **unrelated** (including unlikely or not).

14.4 Investigator Reporting Responsibilities to the Sponsor

14.4.1 Adverse Events

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

14.4.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) will be reported on the Safety Expedited Report Form (SERF) and sent to the Sanaria, Inc. Regulatory Affairs Office by fax or e-mail attachment within 3 business day after the clinical site becomes aware of the event. Deaths and immediately life-threatening SAEs will be reported within 1 business day after the clinical site becomes aware of the event.

Sanaria Inc.
SAE Fax: 240-306-0596

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

2. Thomas L Richie, M.D., Ph.D.
Tel: 240-403-2727 (office)

Tel: 301-466-7943 (mobile)
Email: trichie@sanaria.com

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

In Mali – the clinical site investigator will also notify the LMIV PI and the site medical monitor in Mali by email, fax, or telephone within 1 working day of notification of an SAE occurrence.

LMIV Contact Information:
Patrick Duffy, MD
Tel: (301) 761-5089
Fax: (301) 480 1962
Email: Patrick.Duffy@nih.gov

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

14.4.3 Unanticipated Problems

All UPs that are also AEs will be reported to the IND Sponsor on the NIH Reportable Events Form sent by fax or e-mail 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.

14.4.4 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the IND Sponsor via fax or email within 3 business days from the site awareness of the pregnancy.
Pregnancy outcome data (e.g., delivery outcome, spontaneous, or elective termination of the pregnancy) will be reported to the IND Sponsor within 3 business days of the site's awareness of the outcome on a protocol-specified form.
In the event of pregnancy, the following steps will be taken:

- Discontinue the study agent
- If prior to the 3rd vaccination or within 4 weeks post 3rd or 4th (booster) vaccination, withdrawn from the study but continue in follow-up for safety.
- If pregnancy occurs more than 4 weeks after the 3rd vaccination, participants will be invited to continue in a study on a modified research study schedule with decreased

blood draw volumes (**Appendix A**)
- Report to IND Sponsor
- PI will discuss with Sponsor regarding the timing of the pregnancy from last dose of PfSPZ Challenge to determine whether treatment is warranted, appropriate treatment per standard Malian ministry of health guidelines will be employed if needed
- Report to FMPOS Ethics Committee as an informational item
- Report to Data and Safety Monitoring Board (DSMB), Sponsor Medical Monitor, and Site Medical Monitor
- Advice research subject to notify the obstetrician of study product exposure (infectious SPZ, chloroquine, PYR, artemether, lumefantrine).

14.5 Reporting Procedures to the NIH IRB and FMPOS EC

14.5.1 Reporting to the NIH IRB

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

14.5.2 Reporting to the FMPOS EC

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

14.6 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of the subject (or AEs related to study procedures after signing the informed consent and before enrollment) 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 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 electronic record and the SERF.
SAEs that occur after the study follow-up period that are reported to and assessed by the Investigator to be possibly, probably, or definitely related must be reported to the IND Sponsor as described above.

14.7 Sponsor's Reporting Responsibilities

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA. Sanaria Inc. will also report any relevant safety findings, including AEs that are also UPs, to other clinical trial sites where related Sanaria products are being studied.
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, using data provided by the PI.

14.8 Pausing Criteria for Entire Study Population

The PI will closely monitor 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 PfSPZ-CVac (chloroquine) or PfSPZ-CVac (pyrimethamine) components.

14.8.1 Parasitemia and Malaria Symptoms

LMIV has conducted two clinical trials of PfSPZ-CVac (pyrimethamine) immunization in malaria naïve participants (NIAID protocol #15-I-0169 and #17-I-0067) in which patent parasitemia (positive blood smear) or subpatent parasitemia (qPCR positive) was not detected in any PYR participant during immunization. Subjects enrolled in the chloroquine *arms* did have episodes of both patent and subpatent parasitemia at the expected times and showing the expected densities, and no subjects were re-treated or withdrawn from the study due to these expected episodes of transient parasitemia.

During the **PYR pilot (*Arms 1a, 2a, 5a, 6a*)**, the participants will be monitored closely with sBS in real time from 7-12 days after vaccination. A positive blood smear or a diagnosis of clinical malaria per Malian ministry of health guidelines (presence of symptoms + positive malaria diagnostic test) will result in treatment with standard care, artemether/lumefantrine regimen. A positive blood smear or diagnosis of clinical malaria will result in pausing of the individual participant in the pilot phase only. A positive malaria blood smear during PfSPZ-CVac immunization pilot phase will therefore not necessarily provide a pause for the study as a whole, nor will it be reported as an UP. However, if a positive blood smear occurs, the subject's information, including results and any additional clinical or laboratory information will be sent with a Notification Report to the IND Sponsor for review.

During the **main study and booster phase, in the vaccinated PYR Arms** (***Arms 1b/5b, 2b/6b)***, the participants will be monitored closely clinically daily from 7-9 days after vaccination. Blood smears and malaria qPCRs will be collected but will be analyzed retrospectively. If a participant is symptomatic, the blood smear will be read and reported in real time. A diagnosis of clinical malaria per Malian ministry of health guidelines (presence of symptoms + positive malaria diagnostic test) will result in treatment with standard care. Diagnosis of clinical malaria will **not** result in pausing of the individual participant in the main study phase, but the information will be stored and considered during the analysis at the end of the study. A positive malaria blood smear during PfSPZ-CVac immunization will therefore not necessarily provide a pause for the study as a whole, nor will it be reported as an UP.
The study may pause for further review during the pilot if patent parasitemia is detected in any subjects in *Arms 5a or 6a* as outlined in **Section 3.2** and **Figure 7**.

In summary, ***individual participants*** in **PYR** arms will be treated per standard care in the pilot phase if any of the following occurs:

1. A positive blood smear
2. Diagnosis of clinical malaria
3. At the PI's discretion (for example, if non-compliance with PYR ingestion is suspected, or if concerning signs or symptoms develop)

For **vaccinated chloroquine only arms** (***Arms 3b)*****,** participants receiving chloroquine may present with patent parasitemia as has been seen in other PfSPZ-CVac (chloroquine) and CPS with chloroquine studies during the immunization phase, including positive blood smears. In previous studies (NIAID protocol #15-I-0169, #17-I-0067), most participants in chloroquine only arm did not develop positive blood smears although they did have subpatent parasitemia as documented by positive LMIV qPCR assay. However, in the proposed study, due to higher doses of PfSPZ Challenge, a positive blood smear may occur. Therefore, for *Arm 3*, a positive blood smear during PfSPZ-CVac (chloroquine) immunization will not necessarily result in pausing for an individual participant or the study as a whole, nor will it be reported as an UP.

Individual subjects in the chloroquine arms will be re-treated with another antimalarial medication (artemether/lumefantrine) and withdrawn if any of the following occurs:

1. A positive blood smear outside of the range of expected peak parasitemia (on or after day 12 post PfSPZ Challenge) regardless of symptoms (maximum expected peak parasitemia is 7-9 days post PfSPZ Challenge).
2. A subject with a positive blood smear at any time point and Grade 3 or greater symptoms (NOT laboratory values) lasting longer than 48 hours despite adequate symptomatic management (e.g. non-steroidal anti-inflammatory drugs)
3. At the PI's discretion (for example, if non-compliance with chloroquine ingestion is suspected, or if concerning signs or symptoms develop)

If a positive blood smear occurs during the pilot phase (*Arm 3a*), the subject's information, including blood smear results and any additional clinical or laboratory information will be sent with a Notification Report to the IND Sponsor for review. The Sponsor may additionally notify the Chair of the DSMB for evaluation and consequent further review by the DSMB for determination if the subject's patent parasitemia is clinically significant and warrants re-treatment. The DSMB and the IND Sponsor may recommend close clinical follow-up but no immediate re-treatment, or may determine that the findings warrant re-treatment with a back-up antimalarial regimen.

The following criteria will be used to define unacceptable parasitemia results and will result in an immediate pause of further administration of any PfSPZ Challenge to an ***entire study group*** pending review by the DSMB to determine if the study should be terminated, modified, or continued:

- Two (in the pilot)/ four (in the main study) or more subjects experience a positive blood smear at any time point associated with Grade 3 symptom(s) (NOT laboratory values) lasting longer than 48 hours despite adequate symptomatic management (e.g. non-steroidal anti-inflammatory drugs) that is determined to be possibly, probably or definitely related to PfSPZ Challenge

  OR

- Four or more subjects total (pilot and main study) experience positive blood smears outside of the expected peak parasitemia (on or after day 12 post PfSPZ Challenge) in *chloroquine arms* that is determined to be possibly, probably or definitely related to PfSPZ Challenge

In addition to considering the optimal clinical decisions for the subject, the Sponsor and the DSMB will determine if the event impacts the remainder of the study participants and whether any protocol procedures should be modified.

14.8.2 Reactogenicity

If a study product (PfSPZ Challenge, chloroquine, PYR) 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 IRB.

The following criteria will be used to define unacceptable reactogenicity and will result in an immediate pause of further administration of any component of the investigational product to an ***entire study group*** pending review by the DSMB to determine if the study should be terminated, modified, or continued:

- One or more subjects experience an SAE that is determined to be possibly, probably or definitely related to PfSPZ Challenge, chloroquine, PYR, **or**
- One or more subjects experience a hypersensitivity reaction that is probably or definitely related to PfSPZ Challenge **or**
- Any serious clinical illness occurs that is not explained by a diagnosis that is unrelated or unlikely related to study product **or**
- Two (Pilot study)/Four (Main study) or more subjects experiencing the same solicited AE determined to be possibly, probably or definitely related that persists at Grade 3 or higher for >48 hours with adequate symptoms management during the PfSPZ-CVac immunization phase of the study **or**
- Two (Pilot study)/Four (Main study) or more subjects experiencing a similar unsolicited Grade 3 event or a Grade 4 event that is determined to be possibly, probably or definitely related to the study product **or**
- Any safety issue that the study PI or IND Sponsor determines should pause the study

The IRB, FMPOS EC, 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, IRB, FMPOS EC, the IND Sponsor, the FDA, and other regulatory authorities may also result in suspension of further administration of immunizations at the clinical site (in case of the DSMB, a recommendation of suspension to the Sponsor). The FDA, other regulatory authorities, and the study Sponsor(s) retain the authority to suspend additional enrollment and administration of immunizations for the entire study as applicable.

14.8.3 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 1 business day to the IND Sponsor by fax or email.

The site investigator will inform the LMIV PI, the ISM, and the local IRB that a pausing rule has been met according to their requirements. The LMIV PI will notify the 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.

14.8.4 Resumption of a Paused Study

The IND Sponsor, in collaboration with the PI and the DSMB will determine if it is safe to resume the study. The IND Sponsor will notify the PI 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. The NIH IRB(s) will also be notified.

14.9 Pausing Criteria for a Subject or Group

The decision to pause administration of the study agent(s) for a single participant or for all participants in a specific group requires discontinuation of study agent administrated for the study participant(s) or group until a decision is made whether or not to continue study agent administration.
The pausing criteria for ***a single participant*** or ***for the subjects in a specific arm in this study*** include:

- A subject experiences an SAE or ≥ two Grade 3 or greater AEs that are unexpected (as determined by the IND Sponsor) and lasting longer than 48 hours despite adequate symptomatic management (e.g. non-steroidal anti-inflammatory drugs) that is determined to be possibly, probably or definitely related to a study agent; **or**
- Any safety issue that the Site Investigator determines should pause administration of the study agent to a single participant or to all participants in a specific group.

The IND Sponsor, in collaboration with the PI, may also pause for an individual participant or entire group if a safety concern is identified during routine aggregate data analysis.

14.9.1 Reporting of Pausing for a Participant or Group

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 1 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.

14.9.2 Resumption of a Paused Study

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/group. 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.

14.10 Withdrawal Criteria for an Individual Participant

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. *Developed 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 study products (other than Grade 3 local reactions lasting <72 hours, systemic reactions lasting <24 hours or those that occur 7-10 days after PfSPZ Challenge and assessed to be related to induced malaria lasting <48 hours) will result in withdrawal of the subject from further vaccinations. Subjects may also be withdrawn for any AE that would cause continued participation in the study to not be in the best interest of the subject, as per the investigator's judgment. Any subject who is withdrawn from the study 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. *HIV/Hepatitis Infection* – If a subject acquires HIV or hepatitis infections during the course of the study (either by self reporting and confirmed by study team or by repeat testing for clinical evaluation), then they meet exclusion criteria for the study. The participant will be removed from further scheduled research procedures but will continue to be followed for safety. The event will be reported as an SAE as outlined in **Section 14**.
6. *Pregnancy* – If the subject becomes pregnant during the course of the study, they will be withdrawn for safety purposes if the pregnancy happens prior to the third vaccination or within 4 weeks of receipt of third or fourth (booster) vaccination. If the pregnancy happens more than 4 weeks after the third or fourth (booster) vaccination, the participant will be invited to continue with study participation following a modified study schedule. Pregnant participants will be followed for the course of the pregnancy until pregnancy outcomes are known.
7. *Inability to tolerate study product* – inability to tolerate any component of the study product individually or in combination (chloroquine, PYR, PfSPZ Challenge).
8. *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 database. Any subject who has received at least 1 dose of PfSPZ Challenge will be strongly encouraged to remain in the safety evaluation for the duration of the study (followed at least one more visit post last PfSPZ Challenge). In particular, rigorous follow-up for the development of malaria infection including the institution of presumptive treatment may be required in order to assure the subject's safety. The subject's data will be included in the safety and immunogenicity

analysis. If a subject fails to complete all planned PfSPZ Challenge administration 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.

14.11 Replacement of Withdrawn Subjects

Subjects who have received at least 1 vaccination and who withdraw or are terminated from the study prior to completion will not be replaced. Subjects withdrawn before the first vaccination will be replaced.

14.12 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 SOPs and submitted to the study statistician for formal unblinding.
If emergency unblinding is required, the PI or designee will contact the study statistician or study pharmacist for unblinding. The Sponsor will be informed within one business day that the unblinding was necessary and a submitted "Unblinding Report" will be provided within 2 business days.
Subjects who are unblinded will be encouraged to remain in the study to be followed for safety.

For subjects who do not wish to participate in the booster phase, scheduled unblinding will occur on or about Study Day 238 at the completion of the subject's final visit as already outlined in **Appendix A**. For subjects who do enroll in the booster phase, scheduled unblinding will occur on or about study day 569. All primary and secondary endpoints will have been completed at this time for the arm of the study in which the participant is enrolled. Individuals directly continuing AE assessment or performing assays for exploratory endpoints will remain blinded to individual randomization until assessment or assays are completed.

As noted in Section 1.5.1.3, group unblinding for statistical analysis of blood smear results from the first year of the study (prior to the booster dose) will occur approximately 4 months following receipt of the booster vaccine dose, as long as individual subject blinding can be maintained (as determined by the unblinded statistician). The data will be reviewed by study staff who have no direct contact with study participants nor conduct clinical assessments. This information will only be used to determine if enough data supports moving forward with the pyrimethamine comparator arms on an accelerated vaccination schedule in a future planned trial.

14.13 Safety Oversight

14.13.1 Independent Safety Monitor (ISM) in Mali

An independent safety monitor 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 AEs 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. The ISM will be a voting member of the DSMB.

14.13.2 Data and Safety Monitoring Board (DSMB)

As agreed with Office of Clinical Research Policy and Regulatory Operations (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 data to evaluate the safety, efficacy, study progress, and conduct of the study, these reviews to be conducted during scheduled and ad hoc meetings hosted by the IND Sponsor. The six scheduled meetings are: prior to initiation, in the first year; between the pilot and main phase, between the first and the second vaccination in the main phase, after completion of main phase during the malaria follow up period. In the subsequent year of the study for the CQ arms, additional meetings will be scheduled between the first and second vaccination and at close-out, as outlined in **Figure 7** and **Figure 9**. In addition, the DSMB will review data from the main phase of the study prior to the administration of the booster vaccination. The PI will generate safety reports for review by the IND Sponsor for all but the first meeting; these reports will be submitted to the DSMB prior to the meeting for DSMB review. The board will additionally convene on an ad hoc basis as needed, and will issue recommendations concerning continuation, modification, or termination of the study.

All SAEs and UPs will be reported by the Sponsor to the DSMB. The Sponsor will also notify the DSMB at the time pausing criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The Sponsor will initially consult with the DSMB chair with regard to these events, to obtain recommendations whether an ad hoc meeting should be called.

The PI will submit written DSMB summary reports with recommendations to the IRB(s).

15 Clinical Monitoring

15.1 Site Monitoring Plan

As per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use- Good Clinical Practice (ICH-GCP) guideline 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study Sponsor. OCRPRO will provide oversight and monitor the compliance of this trial. 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 site to

monitor aspects of the trial in accordance with appropriate regulations. The objectives of a monitoring visit will be:

- to verify the prompt reporting of all monitored data points, and prompt reporting of all SAEs
- to check the existence of signed informed consent documents and documentation of the ICF process for each monitored subject
- to compare individual subject's records (e.g. CRFs, electronic data) to the source documents (supporting data, laboratory specimen records, clinical notes)
- to ensure the investigators are in compliance with the protocol

The monitors will also inspect the clinical site's regulatory files to ensure that applicable regulatory requirements (FDA, Office for Human Research Protections [OHRP]) and ICH guidelines are being obeyed. During the monitoring visits, the PI and/or designated study staff will be available to discuss the study. The site PI will provide direct access and allow the study monitors, LMIV, the IND Sponsor, and regulatory authorities to access all study-related documents.

A specific monitoring plan will be discussed with the PI, study staff, and IND Sponsor 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.

Quality control procedures will be implemented beginning with the data entry system, and data quality control checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

16 Statistical Considerations

16.1 General Pilot Study

PfSPZ-CVac (pyrimethamine) groups: The pilot study will investigate whether 75 mg of PYR administered either concurrently (day 0) with PfSPZ injection or on day 2&3 post injection will prevent breakthrough parasitemia in all subjects following DVI of $4.0x10^5$ PfSPZ. The pilot may also explore whether administration of 75mg of PYR on either day 0 or on day 2&3 with $3.0x10^5$ PfSPZ may prevent parasitemia if there is breakthrough with higher dose of PfSPZ.

For the PfSPZ-CVac (chloroquine) groups: The pilot study will investigate whether the $4.0x10^5$ dose of PfSPZ Challenge is safe and well tolerated in malaria-exposed adults or whether NSAIDS are needed to improve tolerability.

16.2 General Main Study and Booster Study

The main phase and booster phase is designed to investigate the safety profile of the selected PfSPZ Challenge regimen with concurrent chemoprophylaxis with PYR or chloroquine in healthy adults. As well, we wish to estimate protective efficacy against natural malaria transmission, starting two weeks after the third vaccination. We will explore whether the timing of the PYR and dose of PfSPZ Challenge can be adjusted to occur more quickly after PfSPZ Challenge without impacting the protective efficacy.

16.3 Primary Endpoints:

Safety

Pilot Phase

- Incidence of positive sBS occurring after PfSPZ-CVac immunization starting on day 7 post DVI (*Arms 1a, 2a, 5a, 6a*).
- Incidence and severity of local and systemic grade 3 signs or symptoms lasting more than 48 hours despite adequate management and serious adverse events (SAEs) occurring after PfSPZ-CVac DVI (*Arm 3a)*

Main Phase and Booster Phase

- Incidence and severity of local and systemic adverse events (AEs) and serious adverse events (SAEs) occurring after PfSPZ-CVac immunization. (*Arms 1b/5b, 2b/6b, 4a, 4b)*

- Incidence of clinical malaria diagnosis occurring after PfSPZ-CVac immunization as defined by the occurrence of grade 3 signs or symptoms lasting more than 48 hours despite adequate management. *(Arm 3b, 4c)*

Protective Efficacy

- *P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood starting 2 weeks after the 3rd vaccination for approximately 6 months and then 2 weeks after the 4th vaccination for approximately 6 months for the booster phase. (*Arm 1b/5b, 2b/6b, 3b, 4*)

Pyrimethamine regimen efficacy and Immunogenicity

- *P. falciparum* blood stage infection defined as detection of *P. falciparum* parasites by qPCR following PfSPZ Challenge. (*Arms 1, 2, 5, 6).)*
- Humoral immune responses after PfSPZ-CVac regimens by assessing antibodies to PfSPZ, Pf asexual erythrocytic stages (AES), and specific Pf sporozoite, liver and blood-stage antigens such as CSP, MSP-1, AMA-1 in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups. *(All Arms)*
- Cellular immune responses after PfSPZ-CVac regimens to PfSPZ, PfAES, and specific Pf sporozoite, liver and blood-stage antigens, such as CSP, MSP-1, AMA-1, in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups. *(All Arms)*
- Cellular and humoral responses that correlate with protective efficacy

- Prolonged prepatent period and/or reduced density and duration of parasitemia in those developing parasitemia during PfSPZ-CVac immunization *(Arms 1, 2, 3, 5, 6)*
- Comparison of γδ T cells before and after PfSPZ-CVac immunization and malaria infection during transmission season using *ex vivo* whole blood or *in vitro* staining. *(All Arms)*

16.3 Analysis

16.3.1 Analysis of Primary Endpoints

Safety in each arm will be summarized descriptively, using proportions and exact 95% confidence intervals.

16.3.2 Analysis of Secondary Endpoints

The protective efficacy of each PYR experimental arm (*Arms 1b and 2b*) in the main phase will be assessed against the first two control arms, pooled (*Arms 4a and 4b*) using a Holm's adjustment based on an overall one-sided type 1 error rate of .025.

The protective efficacy of the chloroquine experimental arm (*Arm 3b*) in the main phase will be assessed against the final control arm (*Arms 4c*) using an overall one-sided type 1 error rate of .025. This will not be adjusted for multiple comparisons, as it will be be considered a separate study for all practical purposes.

The infection rates over time will be compared using log-rank tests. Fisher's Exact Tests will also be conducted, and both will be reported.

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

16.3.3 Analysis of Exploratory Endpoints

Appropriate methods will be used in exploratory data analysis. All findings will be considered hypothesis generating, rather than evidence of any given pre-specified hypothesis.

16.4 Sample Size and Power Calculations

16.4.1 Power calculations for primary objective (safety): Pilot Phase

Table 17 describes the probability of breakthrough parasitemia, infection, or an adverse event during the pilot phase. For a group of four participants, there is a 76% chance of observing at least 1 event if the true rate of such an event is 30% or more; the probability of observing at least 1 event among four participants is 94% if this same true rate of 50%. If we see one event among 4, we will be 95% confident that the true rate will be between 1% and 81%.

Table 17. Probability of observing 0, 1 and 2 or more break through parasitemia or adverse event among arms of size 4, for different true event rates

True event rate (%)	Pr(0/4)	Pr(1/4)	Pr(2+/4)
1	0.96	0.04	<0.01
5	0.81	0.17	0.01
10	0.66	0.29	0.05
20	0.41	0.41	0.18
30	0.24	0.41	0.435
50	0.06	0.25	0.69

16.4.2 Power calculations for Main Phase and Booster Phase

16.4.2.1 Power calculations for primary objective (safety)

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect serious adverse events (SAEs) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for *Arms 1b and 3b* (n=90), there is a 90% chance of observing at least 1 event if the true rate of such an event is 2.5% or more, and a 90% chance of observing at least 2 events if the true rate is at least 4.5%. For *Arm 2b,* (n=60), there is a 90% chance of observing at least 1 event if the true rate of such an event is 4% or more, and a 90% chance of observing at least 2 events if the true rate is at least 6.3%.
Probabilities of observing 0,1, and 2 or more events among Arms of size 90 and 60 are presented in

Table *18*, for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Based on data from existing studies, we expect the number of participants in the booster phase to be similar to that in the main phase. The above sample size considerations should approximately apply to the booster phase.

Table 18. Probability of observing 0, 1 and 2 or more events, among arms of size 90 or 60, for different true event rates

True event rate (%)	Pr(0/90)	Pr(1/90)	Pr(2+/90)	Pr(0/60)	Pr(1/60)	Pr(2+/60)
1	0.40	0.77	0.23	0.55	0.33	0.12
3	0.06	0.24	0.76	0.16	0.30	0.54
5	0.01	0.06	0.94	0.05	0.15	0.80
10	<0.01	<0.01	>0.99	<0.01	0.01	0.99

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. **Error! Reference source not found.Table 19** shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. If none of the 60 participants in an arm in the main study experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 0.06.

Table 19. Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 90 or 60

Observed event rate	95% Confidence interval (%)
0/60	(0.00, 0.06)
1/60	(<0.01, 0.09)
2/60	(<0.01, 0.11)
0/90	(0.00, 0.04)
1/90	(<0.01, 0.06)
2/90	(<0.01, 0.08)

16.4.2.2 Power calculations for secondary objective (efficacy)

Each experimental arm will be compared to the control arm using a Holm's adjustment for multiple comparisons.

Table *20. Sample size calculations for various plausible true event rates among both control and vaccinated subjects* below gives the sample size needed for 80% power to detect a difference using a Fisher's Exact Test or a logrank test and a Bonferroni adjusted alpha of .05/2=.025. **Table 21** gives the power for comparisons of 81/group, allowing 10% loss to follow up. We will have good power to detect a 50% VE for the comparisons of either *Arm 1b* or *3b* to control (*Arm 4*). Since the actual analysis will use a Holm's adjustment, this calculation is conservative. For a given comparison we will have at least 90% power to detect a difference if the true infection rate among the controls is approximately 70% and VE, defined here as 1- the probability of infection in the treatment group divided by the probability of infection in the controls, is at least 50%.

Table 20. Sample size calculations for various plausible true event rates among both control and vaccinated subjects

True infection Rate in		VE	Number needed per group (no LTFU)	
Controls	Vaccinated		Fisher's Exact Test	Log-rank test (Freedman)
.5	.3	.4	123	110
	.25	.5	77	75

	.2	.6	52	52
.6	.36	.4	87	83
	.3	.5	56	55
	.24	.6	39	37

Table 21: Power to detect a difference in efficacy with 81/group (after 10% loss to follow up)

True Infection Rate, controls	True Infection Rate, experimental arm	Vaccine Efficacy (VE)	Power 81/group	Power 54/group
.6	.3	50%	95%	84%
.5	.25	50%	86%	69%

16.5 **Randomization**

Participants in the pilot arms will be randomized into either *Arm 1a, 2a* or *3a*. If additional arms are to be enrolled in the pilot phase, these additional arms (*Arms 5a* and *6a*) will not be randomized unless enrollment into *Arms 5a* and *6a* occurs prior to completion of *Arms 1a, 2a* or *3a 9* (study day 13). If enrollment occurs early, the participants will be randomized into *Arms 5a* and *6a*. They will however be enrolled sequentially as they become eligible to enroll. Subjects will be randomized into enrollment into *Arms 1b/5b, 2b/6b, 4a, and 4b* in the first year. Subjects will be randomized into Arms *3b, and 4c* in a subsequent year. The pilot phase of the study will be open-label, but the main part of the study will be blinded in regard to PfSPZ Challenge or NS administration: subjects and investigators will not be aware of the injection being given, whether *Arm 1b/5b* or *4b*; Arm *2b/6b* or *4b*; or *Arm 3b* or *4c*). The subjects and investigators will be aware of the medication assignments, PYR or chloroquine.

17 **Human Subject Protections and Ethical Obligations**

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

17.1 **Institutional Review Board**

A copy of the protocol, informed consent forms, and other information to be completed by subjects, such as questionnaires, 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 deviations and SAEs as specified in the relevant sections of the protocol.

17.2 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.

The informed consent will be translated into French and administered orally in the native dialect in the case of potential subjects who cannot read. 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. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject's medical record. 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.

17.2.1 Mali Site Community Permission and Individual Informed Consent Process

17.2.1.1 Community Permission

Community permission will be obtained from village elders, family heads, and other community members after explanation and discussion of the study.(Diallo, Doumbo 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. An evaluation checklist is performed to make sure that the study is understood by the subjects before the enrolment.

17.2.1.2 Individual Informed Consent

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

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

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

The Malaria Comprehension Exam will be translated into French and administered orally in the native dialect in the case of potential subjects who cannot read. Study staff will use incorrect answers from the questionnaire to identify those areas of the 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.

17.3 Justification for Exclusion of Children

This study will not enroll children, since safety of the new chloroquine and PYR regimen has not yet been established in adults.

17.4 Justification for the Exclusion of Pregnant Women

This study will not enroll pregnant and/or breastfeeding women since the effects of PfSPZ Challenge on the developing human fetus are unknown. In addition, for the duration of the study, this study will not enroll pregnant women as artemether/lumefantrine are pregnancy category C drugs. The safety and efficacy of artemether/lumefantrine in the treatment of acute, uncomplicated malaria in pregnant women have not been established. Artemether/lumefantrine should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus. Given all subjects in the main study will receive artemether/lumefantrine regardless of the presence of malaria parasites in their blood, the risk of drug is not justified in pregnant women. However, if a woman becomes pregnant after enrollment she may continue in the study for safety follow up or may be invited to continue in the study with modified research procedures if pregnancy happens after the third vaccination. No additional investigational products will be administered.

17.5 Subject Confidentiality

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, including but not limited to, medical records. 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, the FDA, the OHRP, Sanaria Inc., or the Sponsor's designee.

17.6 Risks

Risks to the subjects are associated with venipuncture, malaria infection, PfSPZ Challenge, chloroquine, PYR, and large volume blood drawing. For large blood draws, no more than 80 mL of blood will be drawn at a single time point and the total amount of blood drawn is not to exceed 10.5 mL/Kg or 550 mL whichever is smaller in a 56 day period (as defined as the amount of blood allowed to be drawn under the M95-9: guidelines of blood drawn for research purposes in the NIH Clinical Center).

These study risks are outlined below:

17.6.1 Venipuncture

Risks occasionally associated with venipuncture include pain, bruising, bleeding and infection at the site of venipuncture, lightheadedness, and rarely, syncope.

17.6.2 PfSPZ Challenge administered by DVI

Excluding the malaria symptoms caused by the parasitemia that may follow the injection of non-attenuated PfSPZ (see next section), PfSPZ Challenge as well as other PfSPZ-based products have not caused adverse signs or symptoms, apparently due to the low reactogenicity of purified SPZ, and the asymptomatic nature of liver stage infection. Following the parenteral injection of any vaccine, possible local reactions include transient pain, swelling, erythema, induration, limitation of limb movement, lymphadenopathy, or pruritus at the injection site. Administration of a vaccine may also cause systemic reactions such as fever, chills, headache, fatigue, malaise, myalgia, and joint pain, with some reactions moderate or severe. However, with the exception of mild and transient local (site of administration) reactions, these ARs remain theoretical with respect to PfSPZ-based products, which appear remarkably well tolerated. Similarly, no laboratory abnormalities have been clearly related to the administration of PfSPZ, although in some studies a transient drop in peripheral lymphocyte counts has been documented, likely resulting from margination, diapedesis into interstitial tissues and possibly migration of mononuclear cells to central organs such as the liver, in response to the antigenic stimulus.

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

Miscarriages frequently occur without known causes and although it is unlikely the vaccine caused the miscarriage, the temporal relationship meant this was a possibility. All women of child-bearing age are required to take birth control measures as specified within the protocol and/or consent form to avoid getting pregnant while participating in trials of PfSPZ-based products. In this protocol, volunteers have to use two forms of contraception and must have started a hormonal method at least 21 days prior to receipt of first PfSPZ Challenge injection.

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

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

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

17.6.3 Malaria Symptoms during immunization with PfSPZ-CVac (chloroquine)

Research subjects immunized with PfSPZ-CVac (chloroquine) may experience the usual, well-known side effects of chloroquine, which are discussed in a following section (see below). Other than the side effects of chloroquine, the adverse event profile associated with PfSPZ, as discussed in the preceding section, is benign. However, this changes after six days, due to the exiting of the parasites from the liver into the blood. In malaria naïve participants, there follows a 3 to 4-day period of transient parasitemia that occurs 6-10 days after injection, reaching maximum density on day 7, 8 or 9. Parasitemia then falls quickly, as the parasites are exposed to toxic levels of chloroquine, and are rapidly killed. In the Tübingen TÜCHMI-002 trial, the highest density recorded after administering $5.12x10^4$ PfSPZ of PfSPZ Challenge by DVI was 36.4 parasites/µL. The geometric mean density in all 9 volunteers after the first dose of PfSPZ Challenge was 15.7 parasites/µL. These densities are high enough to cause symptoms in some malaria-naïve individuals, since the symptom threshold is typically crossed at densities of 5-15 parasites/µL, although others may not experience symptoms until parasite densities exceed 50 parasites/µL. The individual with a density of 36.4 parasites/µL in the Tübingen trial did experience transient grade 2 (moderate) symptoms of malaria, which rapidly resolved without intervention. In the second PfSPZ-CVac trial that we are conducting at the NIH (NIAID protocol #17-I-0067), a 4-fold higher dose of PfSPZ was be used, and signs and symptoms of malaria were common, including Grade 3 signs and symptoms, and there were also several subjects with positive thick blood smears. The symptoms quickly resolved and could be prevented almost entirely by presumptive treatment with ibuprofen or another NSAID. Parasite densities fall precipitously during this period, and when highly sensitive quantitative polymerase chain reaction (qPCR) is used for detection, parasite densities fall were very low by day 10 (~ 0.01 parasite/µL) or were undetectable. Any residual low readings on

qPCR appear to represent detection of nucleic acid from non-viable parasites, since recrudescent infections have not occurred in these individuals.

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

To assure the proper administration of CQ to all volunteers, directly observed treatment (DOT) will be done, including oral examination to assure that the tablets have been swallowed. Equally important to assure safety is to monitor for signs and symptoms of parasitemia after immunization, especially days 7-10 after each injection of PfSPZ Challenge as outlined in **Appendix A**. If signs or symptoms likely caused by parasitemia occur, they will be followed closely. Thick blood smears will be collected but read in real time if the participant is symptomatic. If needed (see discussion earlier in the protocol), re-treatment will be instituted with another antimalarial drug or drug combination if clinically indicated.

Interestingly, despite using the same dose of PfSPZ Challenge that induced symptomatic parasitemia in these studies, malaria-related side effects did not occur at all in the two trials where PfSPZ-CVac (chloroquine) has been tested in malaria exposed adults in Africa, one performed in Mali and one in Equatorial Guinea (discussed in detail earlier in the protocol). This is almost certainly due to naturally acquired immunity, which has developed in these individuals after years of exposure to repeated malaria infections. Because the current study will be done in the same population – malaria-exposed African adults – we do not expect significant ARs, even using a dose of PfSPZ Challenge ($4.0x10^5$ PfSPZ) that is nearly twice as high as that studied in the prior trial in Mali ($2.048x10^5$ PfSPZ).

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

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

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

In summary, we do not expect the study subjects to experience signs and symptoms of malaria due to naturally acquired immunity, but because $4.0x10^5$ PfSPZ is the largest dose of PfSPZ Challenge yet administered to humans, the study subjects will be followed closely during the period of transient parasitemia, including symptom checks and blood smears, and retrospective measurement of parasite densities using qPCR.

Immunization with PfSPZ-CVac (pyrimethamine) should not lead to parasitemia. However, this could occur during this trial, as the optimal doses of PfSPZ and PYR are worked out. For this reason, the study subjects in the PYR arms will also be monitored closely starting day 6 after injection of PfSPZ Challenge, with sBS performed daily (qPCRs performed retrospectively) from 6-12 days post injection after first vaccination and 7-9 days post injection after subsequent vaccinations to permit early detection of any breakthrough parasitemia. If parasitemia is detected and clinical malaria is diagnosed per Malian ministry of health guidelines, they will be treated immediately. Otherwise, participants in the pilot phase will be treated for malaria at the end of their study participation.

17.6.4 Medications used in the study

The antimalarial medications administered in the study (PYR, chloroquine, artemether/lumefantrine) are generally well tolerated in the dosing regimens to be used, although common side effects (e.g., nausea, vomiting, diarrhea, headaches, sleep disturbance) are anticipated to occur in some subjects. Potential subjects with known sensitivity or contraindications to the antimalarials administered in this study are excluded from participation. Subjects in the study will be closely monitored for adverse drug reactions and where possible, attempts will be made to minimize anticipated side effects (e.g. dosing

of medications with food). Specific toxicities for PYR, chloroquine and artemether/lumefantrine are noted below and further outlined in the package insert for each drug.

17.6.4.1 Chloroquine Phosphate (CQ)

The most commonly reported side effects of chloroquine dosing include gastrointestinal disturbance, headache, dizziness, blurred vision, insomnia, tinnitus, and pruritus, but generally these effects do not require discontinuation of the drug. High doses of chloroquine, as used to treat rheumatic diseases (>3. 5 mg/kg/day over 1 year or more), have been associated with retinopathy, although generally requires a cumulative dose exceeding 100 grams (Salako 1984) and thus is extremely unlikely at doses used for routine weekly malaria prophylaxis especially for the limited duration in this study. Chloroquine is reported to exacerbate psoriasis; therefore, subjects with a history of psoriasis are excluded. Rarely, chloroquine may cause seizures or life-threatening allergic reactions. If these or other ARs known to be associated with chloroquine occur, even if they are serious (leading to hospitalization), this will not mandate pausing the study. Rather, chloroquine will be immediately discontinued in the affected study subject, any further treatment needed will be given, and no further PfSPZ Challenge injections will be administered.

In a recent study recently completed by LMIV here at the NIH (NIAID protocol # 15-I-0169) using a combination of the live malaria parasites, PYR and chloroquine, one volunteer developed a serious adverse event (SAE) after the third injection. The symptoms included confusion, dizziness, headache, and nausea requiring a short hospital stay to find out the cause of the volunteer's symptoms. The volunteer was treated with an antiviral medication and recovered quickly and fully. It was suspected that the volunteer either had a virus infection of the brain or had possibly developed a rare side effect of chloroquine. Chloroquine is a safe FDA approved medication but there is very low risk of neurological side effects. Volunteers who take chloroquine will be asked about these types of symptoms frequently and at all clinic visits.

In an ongoing LMIV PfSPZ-CVac study at NIH (NIAID protocol # 17-I-0067) in which some participants are receiving chloroquine, one participant presented with acute change in mental status. This was later diagnosed to be due to acute anticholinergic poisoning after ingesting seeds of a *Datura stramonium* plant for recreational purposes. This episode was deemed to also be possibly related to chloroquine due to timing as it is unclear whether concurrent consumption with chloroquine may potentiate the effects of this plant.

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

17.6.4.2 Pyrimethamine

The most commonly reported side effects of PYR dosing include vomiting and anorexia but generally these effects do not require discontinuation of the drug and can be minimized by giving the medication with food. These sides effects may increase with PYR dose but usually disappears promptly upon reduction of dosage. Prolonged and higher doses of PYR, as used to treat toxoplasmosis, may produce megaloblastic anemia, leukopenia, thrombocytopenia, pancytopenia, atrophic glossitis, hematuria, and disorders of cardiac rhythms though this is

extremely unlikely at doses used for this study. Hematologic effects, however, may also occur at low doses in certain individuals, thus hematological parameters will be closely monitored.

PYR has occasionally been associated with severe hypersensitivity reactions (such as Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme, and anaphylaxis), and hyperphenylalaninemia, can occur particularly when PYR is administered concomitantly with a sulfonamide. The approved dosage of PYR for treatment of toxoplasmosis can be higher than utilized for malaria treatment or chemoprophylaxis (75-100 mg per day) and given for prolonged periods of time (weeks to months), usually in conjunction with sulfadoxine and folinic acid. Chronic high doses used in treatment of toxoplasmosis may produce megaloblastic anemia, leukopenia, thrombocytopenia, pancytopenia, atrophic glossitis, hematuria, and disorders of cardiac rhythm.

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

17.6.4.3 Artemether/Lumefantrine

For artemether/lumefantrine the most commonly reported side effects in adults are: headache, anorexia, dizziness, asthenia, arthralgia and myalgia. Generally, these effects do not require discontinuation of the drug.
Artemether/lumefantrine has an acceptable safety profile. Individuals who may have any contraindication for the use of this drug (e.g. prolonged QTc or taking other medications that can prolong QTc, history of myocardial infarction) will be excluded at screening. The most common side effects (i.e., >30%) in adults are: headache, anorexia, dizziness, asthenia, arthralgia, and myalgia. Discontinuation of artemether/lumefantrine due to AE is rare (0.2%) in adults. Rare but serious hypersensitivity reactions (urticarial and angioedema) and skin reactions (bullous eruption) have been reported post marketing.
Artemether/lumefantrine is a Category C pregnancy drug. Thus, all female participants will undergo pregnancy testing prior to receipt of the investigational dose of artemether/lumefantrine at the start of the study. Also, per the package insert, artemether/lumefantrine may decrease the efficacy of hormonal birth control, so female volunteers who are on hormonal birth control will be counseled about back up pregnancy prevention methods.
For complete artemether/lumefantrine safety information, including less commonly reported side effects, please refer to the Package Insert for Coartem® that is provided.

17.6.4.4 Ibuprofen

Another medication that may be used in the study is ibuprofen or an equivalent nonsteroidal anti-inflammatory drug (NSAIDs). These medications pose very low risk in general and are usually available over the counter. Specific toxicities of ibuprofen are outlined below and further details are available in the provided package insert.

Ibuprofen likewise is not a part of the investigational product/regimen, but will be provided to participants in the chloroquine arm (*Arm 4*) if required to mitigate typical symptoms of malaria infection such as headache, myalgia, fever and chills. The most commonly reported

side effects of ibuprofen include abdominal discomfort, heartburn, nausea, skin rash, dizziness, and tinnitus (ringing in the ears). Less frequent side effects include headache, nervousness, itching, edema (fluid retention), constipation, diarrhea, decreased appetite, flatulence, and vomiting. Ibuprofen has rarely been associated with abnormal hepatic function tests, decreased Cr clearance, leukopenia, thrombocytopenia, allergic rhinitis, anaphylaxis, aseptic meningitis, blurred vision, cardiac arrhythmia, confusion, conjunctivitis, cystitis, depression, drowsiness, duodenal ulcer, erythema multiforme, gastric ulcers, gastritis, gastrointestinal hemorrhage, hallucinations, hearing loss, hematuria, hypertension, insomnia, jaundice, palpitations, pancreatitis, peripheral neuropathy, Stevens-Johnson syndrome, tachycardia, and urticaria.

Note: If an equivalent NSAID is provided, the subject will be informed of the possible side effects.

17.7 Risk to the Community

Malaria is not contagious and cannot be spread by person-to-person contact. Vaccination for this study is designed to occur during the dry season in Mali when malaria transmission and the presence of appropriate mosquito vectors in the community to transmit the parasite to others is low.

Benefits: Subjects will not receive any direct benefit from participation in this study. The effect of the vaccine on long term anti-malarial immunity is unknown at this time. It is hoped that information gained in this study will contribute to the development of a safe and effective malaria vaccine.

17.8 Compensation

Subjects will be given in kind (such as rice and/or millet) or cash equivalent, that can be given in multiple installments, to compensate for the time taken to come to the study clinic for study-related visits (**Table 22**). Preferred compensation will be decided in consultation with village elders, but case-by-case exceptions to receive the cash equivalent may be acceptable. The Mali EC recommends compensating the study subject for their time lost for study procedures. The amount is equivalent to USD $6 for each scheduled visit with blood drawn and equivalent to USD $3 for each scheduled visit without blood drawn. For unscheduled visits that request research labs to be drawn at that time, such as positive blood smear visits during the malaria transmission season, subjects will be compensated USD $6.

Table 22: Compensation during the study

Study Group(s)	Study Activity	US Dollar Equivalent in Rice or Millet Dispensed (Local Currency [CFA]
All Arms (Pilot, Main, & Booster)	Screening & Enrollment	$18 – $24 = 9,684 – 12,912F CFA
All Arms (Pilot & Main)	Completion of Vaccination #1 & follow up	$60-$66 = 32,280F – 35,508F CFA
Main Arms	Completion of Vaccination #2 & follow up	$57-63 = 30,666-33,894F CFA

Main Arms	Completion of Vaccination #3 & follow up	$45 = 24,210F CFA
Main Arms	Natural Human Malaria Infection (NHMI)	$72 = 38,736F CFA
Booster Phase	Completion of Vaccination #4 & follow up	$75 = 30,666F CFA
Booster Phase	Natural Human Malaria Infection (NHMI)	$72 = 38,736F CFA

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

18 Data Handling and Record Keeping

18.1 Data Capture and Management

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

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

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 Revisions**

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

Appendix A: Clinical Evaluation and Laboratory Procedures

Please note: dates of subsequent vaccinations are determined based on the dates of preceding vaccinations rather than on a correlation between Study Day and calendar date.

Appendix A: Clinic and Laboratory Procedures For Arms 1a, 5a

	Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Unscheduled Visit
	Study Day (Pilot Study)	-56	-17	-16	-15	1	2	4	8	9	10	11	12	13	14	15	N/A
	Days post-PfSPZ Cvac	-56	-17	-16	-15	0	1	3	7	8	9	10	11	12	13	14	N/A
	Visit windows (days)		-3	-3	-3	+/-3	0	0	0	0	0	0	0	0	0	0	N/A
		Screen	Enroll & AL dose 1	AL dose	AL dose	PfSPZ CVac #1	Clinic f/u	Clinic f/u	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u/AL dose[3]	AL dose	AL dose/Final Visit	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	X
Interim clinical evaluation			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
Conmed review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PYR Dose						X											
Artemether/Lumefantrine TX Dose (All Arms)			X	X	X				(X)	(X)	(X)	(X)	(X)	X	X	X	X
PfSPZ						X											
LABORATORY PROCEDURES	Designated Laboratory	Tube Type															
Screening/Safety Labs																	
CBC with differential	MRTC CAP/UB	EDTA	2	2			2		2						2		
ALT, Cr		SST	3	3			3		3						3		
Hepatitis B, C, HIV testing		SST	5														
Sickle cell screening (from CBC tube)		EDTA		X													
EKG			X														
Urine dipstick or UA		Urine Container	X														
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X			X										
Malaria Infection Assays																	
Retrospective Research qPCR	MRTC CAP/LMIV	EDTA		0.5			0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Retrospective Peripheral Blood Smear[2]	MRTC	EDTA		0.5			0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Daily total			10	6	0	0	6	0	6	6	1	1	1	1	1	5	0	
Cumulative total Pilot			10	16	16	16	22	22	28	34	35	36	37	38	39	44	44	

[1] Last dose of AL must be given at least 14 days prior to injection

[2] Blood smear will be run Real Time in Symptomatic subjects

[3] AL dosing to occur ONLY after labs collected

Appendix A: Clinic and Laboratory Procedures For Arms 2a, 6a

	Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Unscheduled Visit
	Study Day (Pilot Study)	-56	-17	-16	-15	1	2	3	4	8	9	10	11	12	13	14	15	N/A
	Days post-PfSPZ Cvac	-56	-17	-16	-15	0	1	2	3	7	8	9	10	11	12	13	14	N/A
	Visit windows (days)		-3	-3	-3	+/-3	0	0	0	0	0	0	0	0	0	0	0	N/A
		Screen	Enroll & AL dose [1]	AL dose	AL dose	PfSPZ CVac #1	Clinic f/u	PYR dose	PYR dose	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u/AL dose [3]	AL dose	AL dose/Final Visit	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	X	X
Interim clinical evaluation			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
Conmed review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PYR Dose								X	X									
Artemether/Lumefantrine TX Dose			X	X	X					(X)	(X)	(X)	(X)	(X)	X	X	X	X
PfSPZ						X												
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																
Screening/Safety Labs																		
CBC with differential	MRTC CAP/UB	EDTA	2	2			2			2	2						2		
ALT, Cr		SST	3	3			3			3	3						3		
Hepatitis B, C, HIV testing		SST	5																
Sickle cell screening (from CBC tube)		EDTA		X															
EKG		SST	X																
Urine dipstick or UA		Urine Container	X																
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X			X												
Malaria Infection Assays																			
Retrospective Research qPCR	MRTC CAP/LMIV	EDTA	0.5	0.5			0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5			0.5
Retrospective Peripheral Blood Smear [2]	MRTC	EDTA	0.5	0.5			0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5			0.5
Daily total			11	6	0	0	6	0	0	6	6	1	1	1	1	1	5	0	
Cumulative total Pilot			11	17	17	17	23	23	23	29	35	36	37	38	39	40	45	45	

[1] Last dose of AL must be given at least 14 days prior to injection

[2] Blood smear will be run Real Time in Symptomatic subjects

[3] AL dosing to occur ONLY after labs collected

Appendix A: Clinic and Laboratory Procedures For Arm 3a

	Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Unscheduled Visit
	Study Day (Main Study)	-56	-17	-16	-15	-2	1	2	4	6	8	9	10	11	12	13	14	15	N/A
	Days post-PfSPZ Cvac	-56	-17	-16	-15	-2	0	1	3	5	7	8	9	10	11	12	13	14	N/A
	Visit windows (days)		-3	-3	-3	-1	-1 to +3	0	0	+/-1	0	0	0	0	0	0	+/-2	+/-2	N/A
		Screen	Enroll & AL dose [1, 6]	AL dose	AL dose	CQ dose	PfSPZ CVac #1	Clinic f/u	Clinic f/u	CQ dose	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic f/u/AL dose [3]	AL dose	AL dose/Final Visit	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	X	X	X
Interim clinical evaluation			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
Counsel review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CQ dosing						X				X									
Artemether/Lumefantrine TX Dose (ALL Arms)[6]			X	X	X						(X)	(X)	(X)	(X)	(X)	X	X	X	X
PfSPZ							X												
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																	
Screening/Safety Labs																			
CBC with differential	MRTC CAP/UB	EDTA	2	2					2		2						2		
ALT, Cr		SST	3	3					3		3						3		
Hepatitis B, C, HIV testing		SST	5																
Sickle cell screening (from CBC tube)		EDTA		X															
EKG		Cardiology	X																
Urine dipstick or UA		Urine Container	X																
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X			X	X											
Malaria Infection Assay																			
Retrospective Research qPCR	MRTC CAP/UB	EDTA		0.5				0.5		0.5		0.5	0.5	0.5	0.5	0.5	0.5		0.5
Retrospective Peripheral Blood Smear[2]	MRTC CAP/UB	EDTA		0.5				0.5		0.5		0.5	0.5	0.5	0.5	0.5			0.5
Daily total		10	6	0	0	0	1	0	6	0	6	1	1	1	1	1	5	0	
Study cumulative total		10	16	16	16	16	17	17	23	23	29	30	31	32	33	34	39	39	

[1] Last dose of AL must be given at least 14 days prior to injection

[2] Blood smear will be run Real Time in Symptomatic subjects

[3] AL dosing to occur ONLY after labs collected

[6] In Arms 3a, 3b and 4c, first dose of AL (Study Day -17) should be administered 14 days or more prior to CQ loading dose (Study Day -2)

Appendix A: Clinic and Laboratory Procedures For All Arms [1b, 2b, 3b, 5b, 6b, 4a, 4b, 4c] PfSPZ CVac #1

	Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Unscheduled Visit
	Study Day (Main Study)	-56	-17	-16	-15	-2	1	2	3	4	6	8	9	10	13	15	20	27	N/A
	Days post-PfSPZ Cvac	-56	-17	-16	-15	-2	0	1	2	3	5	7	8	9	12	14	19	26	N/A
	Visit windows (days)		-3 to +10	-3 to +10	-3 to +10	-1	-1 to +3	0	0	0	+/-1	0	0	0	0	+/-2	+/-2	+/-2	N/A
		Screen	Enroll & AL dose[4]	AL dose	AL dose	Arm 3b, 4c Only: CQ dose	PfSPZ CVac #1	Clinic f/u	Arm 2b, 6b, 4b Only: PYR dose	Clinic f/u	Arm 3b, 4c Only: CQ dose	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U	Clinic f/u	Clinic f/u	Arm 3b, 4c Only: CQ dose	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	X	X	X
Interim clinical evaluation			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	X
CQ dosing (Arm 3b, 4cONLY)						X					X				X		X	X	
Ibuprofen dosing (Arm 3b, 4c ONLY)[2]												(X)	(X)						
PYR Dose (Arms 2b, 6b, 4b) ONLY)									X	X									
PYR Dose (Arms 1b, 5b, 4a) ONLY)							X												
Treatment with Artemether/Lumefantrine (All Arms) [6]			X	X	X							(X)	(X)	(X)	(X)				(X)
PfSPZ (Arms 1b, 2b, 3b, 5b, 6b ONLY)[5]							X												
LABORATORY PROCEDURES		Tube Type																	
Screening/Safety Labs																			
CBC with differential	MRTC CAP/UB/LPD	EDTA	2	2				2			2		2				2			
ALT, Cr		SST	3	3				3			3		3				3			
Hepatitis B, C, HIV testing		SST	5																	
Sickle cell screening (from CBC tube)		EDTA		X																
EKG		Cardiology	X																	
Urine dipstick or UA		Urine Container	X																	
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X			X	X												
Malaria Infection Assay																				
Retrospective Research qPCR		EDTA		0.5				0.5			0.5		0.5	0.5	0.5	0.5		0.5		0.5
Retrospective Peripheral Blood Smear[3]		EDTA		0.5				0.5			0.5		0.5	0.5	0.5	0.5				0.5
Research Assays																				
Humoral Assays	LMIV/MRTC	SST		8													4			
Cellular Assays		NaHep		30								20					20			
Transcriptional Assays		PAXgene/ Nucleic Acid Stabilizer		1		1					1	1								
Ex vivo		EDTA (pediatric)		1							1	1					1			1
Daily total		10	46	0	1	0	6	0	0	8	0	28	1	1	1	30	0.5	0	
Study cumulative total		10	56	56	57	57	63	63	63	71	71	99	100	101	102	132	132.5	132.5	

[1] NSAID use in Arm 4b to be determined during pilot study

[2] Blood smear will be run Real Time in Symptomatic subjects

[5] Arms 4a, 4b, 4c will receive Normal Saline injection (not PfSPZ)

[6] In Arms 3a, 3b and 4c, first dose of AL (Study Day -17) should be administered 14 days or more prior to CQ loading dose (Study Day -2)

Appendix A: Clinic and Laboratory Procedures For All Arms [1b, 2b, 3b, 5b, 6b, 4a, 4b, 4c] PfSPZ CVac #2																	
	Clinic visits	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Unscheduled Visit
	Study Day (Main Study)	29	30	31	32	34	36	37	38	41	43	44	45	48	55	N/A
	Days post-PfSPZ Cvac	0	1	2	3	5	7	8	9	12	14	15	16	19	26	N/A
	Visit windows (days)	+/-7	0	0	0	+/-1	0	0	0	+/-1	+/-1	+/-1	+/-1	+/-2	+/-5	N/A
		PfSPZ CVac #2	Clinic F/U	Arm 2b, 6b, 4b Only: PYR dose	Clinic f/u	Arm 3b, 4c Only: CQ dose	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Arm 3b, 4c Only: CQ dose	AL dose[2]	AL dose	AL dose	Arm 3b, 4c Only: CQ dose	Clinic 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	X	X	X	X	X	X	X	X	X	X	X	X	
Interim clinical evaluation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
AE/SAE assessment		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	
CQ dosing (Arm 3b,4c ONLY)						X				X				X	X		
PYR Dose (Arms 2b, 6b, 4b ONLY)				X	X												
PYR Dose (Arms 1b, 5b, 4a ONLY)		X															
Artemether/Lumefantrine TX Dose (All Arms)							(X)	(X)	(X)		X	X	X			(X)	
PfSPZ (Arms 1b, 2b, 3b, 5b, 6b ONLY)[3]		X															
LABORATORY PROCEDURES	Designated Laboratory	Tube Type															
Screening/Safety Labs																	
CBC with differential	MRTC CAP/UB/LPD	EDTA				2		2								2	
ALT, Cr		SST				3		3								3	
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X								X						
Malaria Infection Assays																	
Retrospective Research qPCR		EDTA	0.5					0.5	0.5	0.5							0.5
Retrospective Peripheral Blood Smear[1]		EDTA	0.5					0.5	0.5	0.5							0.5
Research Assays																	
Humoral Assays	LMIV/MRTC	SST									4						
Cellular Assays		NaHep						20								20	
Transcriptional Assays		PAXgene/ Nucleic Acid Stabilizer				1		1			1						
Ex vivo		EDTA				1		1			1					1	1
Daily total		1	0	0	7	0	28	1	1	0	6	0	0	0	26		
Study cumulative total		133.5	133.5	133.5	140.5	140.5	168.5	169.5	170.5	170.5	176.5	176.5	176.5	176.5	202.5		

[1] Blood smear will be run Real Time in Symptomatic subjects

[2] AL dosing to occur AFTER labs collected

[3] Arms 4a, 4b, 4c will receive Normal Saline injection (not PfSPZ)

Appendix A: Clinic and Laboratory Procedures For All Arms [1b, 2b, 3b, 5b, 6b, 4a, 4b, 4c] PfSPZ CVac #3

		Clinic visits	32	33	34	35	36	37	38	39	40	Unscheduled Visit
		Study Day (Main Study)	57	58	59	60	62	64	65	66	71	N/A
		Days post-PfSPZ Cvac	0	1	2	3	5	7	8	9	14	N/A
		Visit windows (days)	+/-5	0	0	0	+/-1	0	0	0	+/-3	N/A
			PfSPZ CVac #3	Clinic F/U	Arm 2b, 6b, 4b Only: PYR dose	Clinic f/u	Arm 3b, 4c Only: CQ dose	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic 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	X	X	X	X	X	X	X
Interim clinical evaluation			X	X	X	X	X	X	X	X	X	X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X
CQ dosing (Arm 3b, 4c ONLY)							X					
PYR Dose (Arms 2b, 6b, 4b ONLY)					X	X						
PYR Dose (Arms 1b, 5b, 4a ONLY)			X									
Artemether/Lumefantrine TX Dose								(X)	(X)	(X)		(X)
PfSPZ (Arms 1b, 2b, 3b, 5b, 6b ONLY)[5]			X									
LABORATORY PROCEDURES	Designated Laboratory	Tube Type										
Screening/Safety Labs												
CBC with differential	MRTC CAP/UB	EDTA				2		2				
ALT, Cr		SST				3		3				
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X									
Malaria Infection Assays												
Retrospective Research qPCR		EDTA	0.5					0.5	0.5	0.5	0.5	0.5
Retrospective Peripheral Blood Smear[1]		EDTA	0.5					0.5	0.5	0.5	0.5	0.5
Research Assays												
Humoral Assays	LMIV/MRTC	SST						4			4	
Cellular Assays		NaHep						20			20	
Transcriptional Assays		PAXgene/ Nucleic Acid Stabilizer				1		1			1	
Ex vivo		EDTA				1		1			1	1
Daily total			1	0	0	7	0	32	1	1	27	
Study cumulative total			203.5	203.5	203.5	210.5	210.5	242.5	243.5	244.5	271.5	

[1]Blood smear will be run Real Time in Symptomatic subjects

[5] Arms 4a, 4b, 4c will receive Normal Saline injection (not PfSPZ)

Appendix A: Clinic and Laboratory Procedures For All Arms [1b, 2b, 3b, 5b, 6b, 4a, 4b, 4c] Natural Human Malaria Infection (NHMI) Surveillance															
		Clinic visits	41	42	43	44	45	46	47	48	49	50	51	52	Unscheduled Visit
		Study Day (Main Study)	84	98	112	126	140	154	168	182	196	210	224	238	N/A
		Days post Vaccine #3	27	41	55	69	83	97	111	125	139	153	167	181	N/A
		Weeks post Vaccine #3	4	6	8	10	12	14	16	18	20	22	24	26	
		Visit windows (days)	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+120	N/A
			Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u Final Unblinding visit	Unscheduled Visit/ Positive BS
CLINICAL PROCEDURES															
Complete medical history/ physical															
Informed consent														X[4]	
Malaria Comprehension Exam															
Pre-test/Post-test HIV counseling															
Pregnancy Prevention Counseling			X												X
Interim clinical evaluation			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X
Artemether/Lumefantrine TX Dose			(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X
LABORATORY PROCEDURES	Designated Laboratory	Tube Type													
Screening/Safety Labs															
CBC with differential	MRTC CAP/UB/LPD	EDTA	2		2			2			2				
ALT, Cr		SST	3		3			3			3				
Malaria Infection Assays															
Retrospective Research qPCR		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
Retrospective Peripheral Blood Smear[1]		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	LMIV/MRTC	SST	4											4	
Cellular Assays[2]		NaHep	30											30	
Transcriptional Assays		PAXgene/ Nucleic Acid Stabilizer	1												
Parasite Purification (CF11)[3]		EDTA	(4)		(4)			(4)			(4)				(4)
Ex vivo		EDTA	1					1						1	(1)
Daily total			42	1	6	1	1	7	1	1	6	1	1	36	
Study cumulative total			313.5	314.5	320.5	321.5	322.5	329.5	330.5	331.5	337.5	338.5	339.5	375.5	

[1] Blood smear will be run Real Time in Symptomatic subjects

[2] If a woman becomes pregnant 4 weeks after the 3rd vaccination and remains in the study, ONLY 5 mL will be drawn if there are no other contraindications for blood draw.

[3] Parasite purification only to be completed if there is a positive blood smear and a sample has not been completed in the previous 28 days

[4] Informed Consent for the Booster phase may be obtained at this visit (Study Day 238)

Appendix A: Clinic and Laboratory Procedures For All Arms [1b, 2b, 3b, 4a, 4b, 4c] PfSPZ CVac #4 (Booster)

	Clinic visits	53	54	55	56	57	58	59	60	61	62	63	64	65	66	Unscheduled Visit
	Study Day (Booster)	314	370	371	372	385	387	388	389	390	392	394	395	396	401	N/A
	Days post-PfSPZ Cvac #4 (Booster)	-56	-17	-16	-15	-2	0[5]	1	2	3	5	7	8	9	14	N/A
	Visit windows (days)		-3 to +10	-3 to +10	-3 to +10	-1	+/-90	0	0	0	+/-1	0	0	0	+/-2	N/A
		Booster Screen	Enroll & AL dose[3]	AL dose	AL dose	Arm 3b, 4c Only: CQ dose	PfSPZ CVac #4 (Booster)	Clinic f/u	Arm 2b, 4b Only: PYR dose	Clinic f/u	Arm 3b, 4c Only: CQ dose	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic f/u	Unscheduled Visit/ Positive BS
CLINICAL PROCEDURES																
Complete medical history/ physical		X														
Informed consent		X[2]														
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
CQ dosing (Arm 3b, 4c ONLY)						X					X					
PYR Dose (Arms 2b, 4b) ONLY)									X	X						
PYR Dose (Arms 1b, 4a) ONLY)							X									
Treatment with Artemether/Lumefantrine (All Arms)[3]			X	X	X							(X)	(X)	(X)		(X)
PfSPZ (Arms 1b, 2b, 3b ONLY)[5]							X									
LABORATORY PROCEDURES	Tube Type															
Screening/Safety Labs																
CBC with differential	EDTA	2	2				2			2		2			2	
ALT, Cr	SST	3	3				3			3		3			3	
Hepatitis B, C, HIV testing[4]	SST	5														
EKG[4] MRTC CAP/UB/LPD	Cardiology	X														
Urine dipstick or UA	Urine Container	X														
Urine/Serum Pregnancy Test (females only)	Urine Container or Lithium Hep	X	X		X	X	X									
Malaria Infection Assay																
Retrospective Research qPCR	EDTA		0.5				0.5			0.5		0.5	0.5	0.5		0.5
Retrospective Peripheral Blood Smear[1]	EDTA		0.5				0.5			0.5		0.5	0.5	0.5		0.5
Research Assays																
Humoral Assays	SST		8												4	
Cellular Assays LMIV/MRTC	NaHep		30									20			20	
Transcriptional Assays	PAXgene/ Nucleic Acid Stabilizer		1		1					1		1				
Ex vivo	EDTA (pediatric)		1							1		1			1	1
Daily total		10	46	0	1	0	6	0	0	8	0	28	1	1	30	
Study cumulative total		385.5	431.5	431.5	432.5	432.5	438.5	438.5	438.5	446.5	446.5	474.5	475.5	476.5	506.5	

[1] Blood smear will be run Real Time in Symptomatic subjects

[2] Informed Consent will be obtained if not already done on Study Day 238

[3] In Arms 3a, 3b and 4c, first dose of AL should be administered 14 days or more prior to CQ loading dose

[4] If clinically indicated

[5] PfSPZ CVac #4 (Booster) will be planned for approximately 11 months after CVac #3, with study days and windows for the Booster Phase calculated from the day the Booster dose is given

Appendix A: Clinic and Laboratory Procedures For All Arms [1b, 2b, 3b, 4a, 4b, 4c] Natural Human Malaria Infection (NHMI) Surveillance Post Cvac #4 (Booster)															
		Clinic visits	67	68	69	70	71	72	73	74	75	76	77	78	Unscheduled Visit
		Study Day (Main Study)	415	429	443	457	471	485	499	513	527	541	555	569	N/A
		Days post Vaccine #4 (Booster)	28	41	56	70	84	98	112	126	140	154	168	182	N/A
		Weeks post Vaccine #4 (Booster)	4	6	8	10	12	14	16	18	20	22	24	26	
		Visit windows (days)	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	N/A
			Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u Final Unblinding visit	Unscheduled Visit / Positive BS
CLINICAL PROCEDURES															
Pregnancy Prevention Counseling			X												X
Interim clinical evaluation			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X
Artemether/Lumefantrine TX Dose			(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X
LABORATORY PROCEDURES	Designated Laboratory	Tube Type													
Screening/Safety Labs															
CBC with differential	MRTC CAP/UB/LPD	EDTA	2		2			2			2				
ALT, Cr		SST	3		3			3			3				
Malaria Infection Assays															
Retrospective Research qPCR		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
Retrospective Peripheral Blood Smear[1]		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	LMIV/MRTC	SST	4											4	
Cellular Assays[2]		NaHep	30											30	
Transcriptional Assays		PAXgene/ Nucleic Acid Stabilizer	1												
Parasite Purification (CF11)[3]		EDTA	(4)		(4)			(4)			(4)				(4)
Ex vivo		EDTA	1					1						1	(0.5)
Daily total			42	1	6	1	1	7	1	1	6	1	1	36	
Study cumulative total			548.5	549.5	555.5	556.5	557.5	564.5	565.5	566.5	572.5	573.5	574.5	610.5	

[1] Blood smear will be run Real Time in Symptomatic subjects

[2] If a woman becomes pregnant 4 weeks after the 3rd vaccination and remains in the study, ONLY 5 mL will be drawn if there are no other contraindications for blood draw.

[3] Parasite purification only to be completed if there is a positive blood smear and a sample has not been completed in the previous 28 days

Appendix B: Toxicity Table

These tables are modified versions of the FDA Toxicity Grading Scale for healthy adult and adolescent subjects enrolled in Preventive Vaccine Clinical Trials to be used to grade adverse events.

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	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 *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	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
Bruising	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	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

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Fever (°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 - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	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
Shortness of breath	Shortness of breath with running or doing more than ordinary effort****	Shortness of breath on doing ordinary effort	Shortness of doing less than ordinary effort	Shortness of breath at rest
Palpitations	No or minimal interference with usual activities	Greater than minimal interference with usual activities or with associated symptoms	Inability to perform usual activities	ER visit or hospitalization
Photosensitivity/ Rash	Localized rash with no medical intervention indicated	Localized rash with medical intervention indicated	Generalized rash with medical intervention indicated	ER visit or hospitalization
Abdominal Pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Anorexia	Transient (< 24 hours) or intermittent anorexia with no or minimal interference with oral intake	Persistent anorexia resulting in decreased oral intake for 24 – 48 hours	Persistent anorexia resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Weight loss	5 to <10% from baseline, nutritional supplement not required	10 to <20% from baseline, nutritional supplement required	≥ 20% from baseline, invasive intervention for nutritional support required	Life-threatening consequences

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Back Pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Chills/Sweats/ Rigors	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Generalized Pruritus	Itching causing no or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Generalized Edema	Localized edema causing no or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Dizziness/Vertigo	Dizziness/vertigo causing minimal interference and above baseline	Dizziness/vertigo causing greater than minimal interference with usual social &	Dizziness/vertigo causing inability to perform usual social & functional activities	Disabling dizziness/ vertigo causing inability to perform basic self-care
Change in Vision	Change in vision causing minimal interference and above baseline	Difficulty seeing causing greater than minimal interference with usual social & functional activities	Difficulty seeing (diplopia, blurriness) causing inability to perform usual social & functional activities	Disabling change in vision (visual field loss, blindness) causing inability to perform basic self-care functions
Sleep disorder (Insomnia, or Difficulty Sleeping, Drowsiness, etc.)	Difficulty sleeping/ Sleep disorder causing minimal interference and above baseline	Sleep disorder/ Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Sleep disorder/ Difficulty sleeping causing inability to perform usual social & functional activities	Disabling sleep disorder/insomnia causing inability to perform basic self-care functions
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

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
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	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
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
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
Muscle weakness	No interference with activity	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Urticaria	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
Tinnitus	Ringing in your ears causing no or minimal interference with usual activities	Greater than minimal interference with usual activities secondary to ringing in your ears	Inability to perform usual activities secondary to ringing in your ears.	ER visit or hospitalization
Peripheral Neuropathy	Tingling in your hands or feet causing no or minimal interference with usual activities	Tingling in your hands or feet causing greater than minimal interference with usual activities	Inability to perform usual activities due to tingling or pain in your hands or feet	ER visit or hospitalization

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Anxiety	No interference with activity	Repeated use of anti-anxiety medication > 24 hours or some interference with activity	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
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

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** 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.
**** Ordinary effort is that of the person himself / herself regards as previous effort of tolerance and usual life style (New York Heart Association definition)

Mali Laboratory AE Grading
Hematology/Chemistry

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

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

Mali Adult Normals
Chemistry

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

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

Hematology

Hematology[1]	Reference Range
Hemoglobin (Female) - gm/dL	9.1 – 13.8
Hemoglobin (Male) - gm/dL	10.8 – 15.8
WBC – 10^3/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
Platelet Count (Male)- 10^3/µL	114 – 335

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

Urine Dip/Urinalysis

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

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

Appendix C: Cardiovascular Risk Assessment

As part of the eligibility determination, subjects will be screened for cardiac risk based on the NHANES I study criteria (Gaziano, Young et al. 2008) and screening electrocardiogram. Results will be documented in the subject source documentation and Cardiac Risk Assessment CRF.

NHANES cardiovascular risk assessment includes the following assessments:

- Evaluation of **risk factors:** Calculated BMI [weight (kg)/height (m^2)], measured Systolic Blood Pressure, smoking status and known diabetes status as reported by the subject on review of medical history
- Evaluation of **5-year cardiovascular risk** for females or males: Low, Moderate, High

Note: subjects under the age of 35 are considered low risk by the NHANES I risk assessment
Only subjects classified as low risk by the NHANES I criteria (green and blue categories below) who have a non-significant ECG, as determined by the study cardiologist, are eligible for the study.

NHANES I Classification for Women

NHANES I Classification for Men

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