Abbreviated Title: PfSPZ Vaccine in Malian Children

FMPOS EC #: N°2021/151/CE/USTTB

Project Assurance: FWA #00001769

Sponsor (Sanaria) Protocol #: MLSPZV5

Version Date: 4.0, 01 December 2022

Title: Phase 2 Trial of Safety, Immunogenicity, and Efficacy against *Plasmodium falciparum* Malaria of PfSPZ Vaccine in Children in Mali

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Drug Name:	PfSPZ Vaccine
Sponsor:	Sanaria Inc
Manufacturer:	Sanaria Inc

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Malaria Research and Training Center
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1. ABBREVIATIONS

AE	Adverse Event
AL	Artemether/lumefantrine
ANC	absolute neutrophil count
ANCOVA	Analysis of Covariance
ATP	According to Protocol
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
Cr	creatinine
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
ECG	electrocardiogram
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HR	hazard ratio
HSA	human serum albumin
IATA	International Air Transport Association
IB	Investigator's Brochure

ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IFA	immunofluorescence assay
IgE	immunoglobulin E
IgG	immunoglobulin G
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISI	inhibition of sporozoite invasion assay
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
LMIV	Laboratory of Malaria Immunology and Vaccinology
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NASBA	nucleic acid sequence-based amplification
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NS	normal saline
OCICB	Office of Cyber Infrastructure and Computational Biology
OHRP	Office for Human Research Protections
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RNA	ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Seasonal Malaria Chemoprophylaxis
SNP	single nucleotide polymorphisms
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
USTTB	University of Sciences, Techniques and Technology, Bamako

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

2. PROTOCOL SUMMARY

2.1 SYNOPSIS

Title: Phase 2 Trial of Safety, Immunogenicity, and Efficacy against *Plasmodium falciparum* Malaria of PfSPZ Vaccine in Children in Mali

Study Description: This is a clinical trial of Sanaria® PfSPZ Vaccine, a non-adjuvanted, live (metabolically active), radiation-attenuated, non-replicating, whole sporozoite (SPZ) vaccine designed to prevent malaria infection caused by *Plasmodium falciparum* (Pf).

In this randomized, double-blind, placebo-controlled trial, approximately 268 (up to 290) healthy children aged 6-10 years will be administered three doses of $9.0x10^5$ Pf sporozoites (PfSPZ) of PfSPZ Vaccine (or placebo) at 1, 8, and 29-days using direct venous inoculation (DVI).

The study is composed of a single cohort with two arms enrolled concurrently:

Arm 1 (PfSPZ Vaccine): (n = ~134) children ages 6 – 10 will receive three doses of PfSPZ Vaccine ($9.0x10^5$ PfSPZ) via direct venous inoculation (DVI) at 1, 8, and 29 days

Arm 2 (normal saline): (n = ~134) children ages 6 – 10 will receive normal saline via DVI at 1, 8, and 29 days

All subjects will receive artemether-lumefantrine (AL) approximately 1- 2 weeks before the first and, if there is evidence of significant malaria transmission in the community or the rainy season begins, a third dose of PfSPZ Vaccine or normal saline for clearance of *Pf* parasitema.

Vaccinated participants and non-immunized placebo controls will be monitored using passive and active case detection for development of Pf *malaria with symptoms* for 26 weeks after V3 through the natural malaria transmission season to estimate vaccine efficacy (VE). Case ascertainment will be prompted by clinical signs and symptoms of acute malaria, with subsequent confirmation of parasitemia by TBS. Participants will be monitored retrospectively for *Pf malaria* (parasitemia) as a secondary endpoint.

Note: for the purposes of this protocol the following definitions apply:

Primary case definition:

Pf *malaria with symptoms* is defined as a positive thick blood smear at a density of >1000 parasites/uL (P/uL) plus:

- Measured axillary temperature ≥ 37.5 degrees Celsius or history of fever (subjective or objective) in the last 24 hours, or,
- Symptoms of malaria:
    - Verbal individual (individual able and willing to answer questions): A verbal individual is considered symptomatic if reporting at the time of evaluation at least two of the following symptoms/symptom groups: headache, chills and/or rigors, malaise and/or fatigue, dizziness and/or light-headedness, myalgias and/or arthralgias; or
    - Non-verbal individual (small child or any individual unable or unwilling to answer questions): A non-verbal individual is considered symptomatic if manifesting at the time of evaluation at least two of the following signs/sign groups: drowsiness, irritability and/or fussiness, inability and/or refusal to eat or drink, prostration; or
- Any individual: Signs of severe malaria (e.g. impairment of consciousness, severe anemia, hemoglobinuria, acute kidney injury, etc.)

<u>Secondary case definition:</u>

Pf *malaria with symptoms* is defined as a positive thick blood smear at a density of > 0 P/uL plus:

- Measured axillary temperature ≥ 37.5 degrees Celsius or history of fever (subjective or objective) in the last 24 hours, or,
- Symptoms of malaria as defined in the primary case definition; or
- Meeting criteria for severe malaria

Pf *malaria* is defined as:

- At least one unambiguous asexual parasite on thick blood smear identified by two independent microscopists after each examining 0.50 µL of blood in a study participant

Objectives:

Primary Objectives:

1. To describe the safety of PfSPZ Vaccine in children with respect to the occurrence of possibly, probably, or definitely related serious adverse events (SAEs).
2. To measure VE against first episode of Pf *malaria with symptoms* by time-to-event analysis.

Secondary Objectives:

1. To assess the safety of PfSPZ Vaccine with respect to the occurrence of unsolicited AEs, laboratory abnormalities, solicited Aes.
2. To measure VE against first episode of Pf *malaria* (parasitemia) with or without associated symptoms by time-to-event analysis.
3. To measure antibody responses to Pf circumsporozoite protein (CSP) and their association with protection.

Exploratory Objectives:

1. To assess the immune response to PfSPZ Vaccine in children.
2. To assess genetic relatedness of the PfSPZ Vaccine parasite strain to malaria infection parasites.
3. To measure VE using other outcome measures such as against all episodes of Pf *malaria with symptoms* and Pf *malaria* rather than against the first episode, using proportional analysis, or using ITT or ATP populations, or severity of malaria infections or hemoglobin concentration.
4. To explore the relationship between detectable parasitemia prior to vaccination and VE.

Endpoints:

Primary Endpoints:

1. Proportion of vaccinees compared to controls experiencing related SAEs from V1 to 26 weeks after V3
2. VE computed as one minus the estimated hazard ratio (HR) (time-to-event analysis) for first episode of Pf *malaria with symptoms* from 2 weeks after V3 to 26 weeks after V3 in the modified ITT (mITT) population.

Secondary Endpoints:

1. Safety and tolerability in children
    - The differences in proportions of vaccinees compared to controls experiencing unsolicited Aes from the time of V1 to 14 days after V3 (or last immunization).

- The differences in proportions of vaccinees compared to controls experiencing laboratory abnormalities from time of V1 to 14 days after V3.
- The differences in proportions of vaccinees compared to controls experiencing solicited Aes during the 7 days following each immunization.

2. VE computed as one minus the estimated hazard ratio (HR) for first episode of Pf *malaria* detected by thick blood smear (TBS), from 2 weeks after V3 to 26 weeks after V3 in the mITT population.
3. Antibody levels to PfCSP by standardized ELISA comparing protected and unprotected vaccinees and controls.

Exploratory Endpoints:

1. Humoral and cellular immune responses to PfSPZ Vaccine
2. Genotyping of peripheral blood Pf parasites
3. Additional measures of VE
   - VE against all episodes (rather than first episode) of Pf *malaria with symptoms* and Pf *malaria* by Cox regression analysis
   - VE computed by proportional (binary) analysis (1 – risk ratio) for first episode of Pf *malaria* detected by TBS, from 2 weeks after V3 to 26 weeks after V3 in the intention-to-treat (ITT), mITT and according to protocol (ATP) populations (whole group and subgroups)
   - VE computed as one minus the estimated HR for first episode of Pf *malaria* detected by TBS, from 2 weeks after V3 to 26 weeks after V3 in the ITT and ATP populations (whole group and subgroups)
   - VE for Pf *malaria* measured by and nucleic acid detection in children from 2 weeks after V3 to 26 weeks after V3 using both (time to event, proportional/binary)
   - VE by both time-to-event and proportional/binary analyses for first episodes of Pf *malaria with symptoms* with
     - parasite density > 500 parasites/μL, or
     - parasite density > 5000 parasites/μL
   - or Pf *malaria with symptoms* characterized by:
     - grade 3 signs or symptoms,
     - associated with fever and convulsions, or
     - hospitalization, or
     - other clinical measures in vaccinees vs. controls
   - Rates of anemia (grade 1, grade 2, grade 3) in vaccinees vs. controls.
4. Retrospective malaria qPCR values taken prior to presumptive AL treatment and vaccination

Study Population:	Approximately 268 (maximum 290) healthy children (6 to10 years of age), resident in Bancoumana and surrounding villages, Mali. Given the potential for dropouts between AL dosing and first vaccinations, up to 290 participants will be dosed with AL prior to first vaccination in order to ensure the target vaccination numbers are met. All participants dosed with AL will also be offered vaccination (or placebo).
Phase:	2
Description of Sites/Facilities Enrolling Participants:	Malaria Research and Training Center (MRTC) research clinic in Bancoumana, Mali
Description of Study Intervention:	Radiation attenuated, aseptic, purified, vialed, cryopreserved, NF54 *Plasmodium falciparum* sporozoites (referred to as PfSPZ Vaccine) produced by Sanaria Inc. will be administered via DVI at dose $9.0x10^5$ PfSPZ at 1, 8, and 29 days Normal saline administered via DVI at 1, 8, and 29 days
Study Duration:	22 months March 2022-July 2022 (screening + enrollment + vaccinations) January 2023 (completion of follow-up) January 2024 (completion of data analyses)
Participant Duration:	Approximately 9 months

2.2 STUDY SCHEMA AND STUDY FLOW DIAGRAM

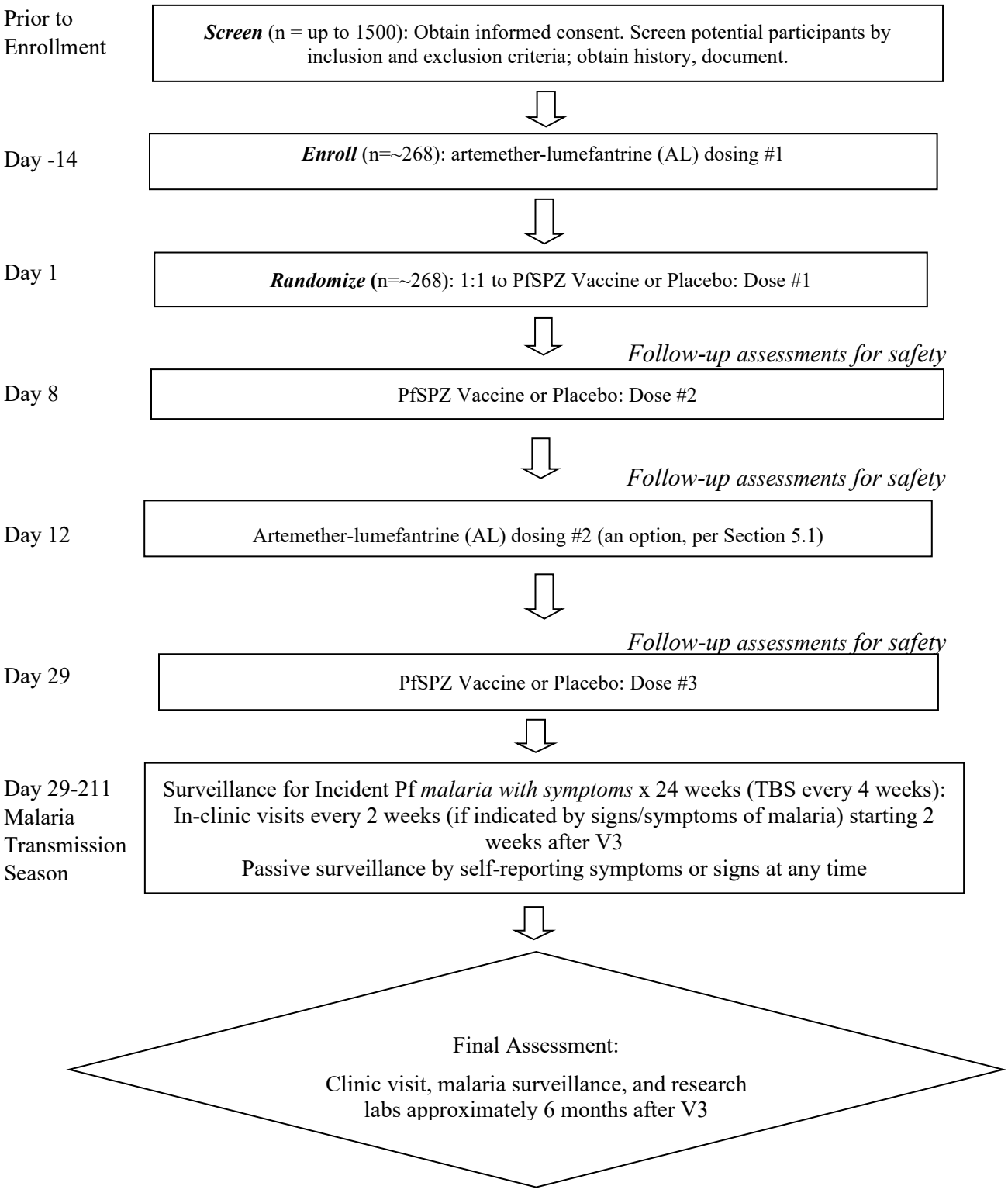
This study will enroll healthy Malian children between 6 and 10 years of age to participate in a randomized, double blind, placebo-controlled study to assess the safety, immunogenicity and protective efficacy of PfSPZ Vaccine. Participants will be immunized with a 3-dose series of 9.0 $x10^5$ PfSPZ of PfSPZ Vaccine or normal saline (placebo) at 1, 8, and 29 days Randomization into the two arms will be in a 1:1 ratio (Erreur ! Source du renvoi introuvable.**)**. The primary follow-up will be for six months. The study flow is shown in **Figure 1.**

Figure 1. Study Schema

Study Weeks	-2	-1	0	1	2	3	4	6	8	10	12	14	16	18	20	22	24	26	30
Vaccinations			V1	V2			V3												
AL dosing	AL				AL														
Follow-up clinic visits								X	X	X	X	X	X	X	X	X	X	X	X

NB: Primary surveillance follow-up visits begin 2 weeks after V3 and occur every other week through week 26 post V3

Figure 2. Study Schema (For details of study visits please see **Section 2.3, Schedule of Activities).**



2.3 SCHEDULE OF ACTIVITIES (SOA)

The schedule of assessment activities is detailed in **Erreur ! Référence non valide pour un signet.** and

Clinic Visits			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Unscheduled Visit
Study Day			-73	-17	-16	-15	1	3	8	10	(12)[M]	(13)[M]	(14)[M]	22	29	32	36	NA
Days post PfSPZ Vaccination #1			-	-	-	-	0	2	7	9	11	12	13	21	28	31	35	NA
Days post last PfSPZ Vaccination			-	-	-	-	0	2	0	2	4	5	6	14	0	3	7	NA
Visit window (days)			→	-24/+10	→	→	0	+/-1	-1/+3	-1/+3	+7/-1	→	→	+/-3	+/-3	+/-1	+/-2	NA
			Screening	Enrollment and AL dosing	AL dose	AL dose	PfSPZ Vac #1	Safety f/u	PfSPZ Vac #2	Safety f/u	AL dosing	AL dose	AL dose	Pre-Vac	PfSPZ Vac #3	Safety f/u	Safety f/u	*Interim Clinical OR Positve f/u visit*
CLINICAL PROCEDURES																		
Complete medical history/physical			X															
Informed consent			X															
Malaria comprehension exam			X															
Pre-test/post-test HIV counseling			X															
Interim clinical evaluation				X	X	X	X	X	X	X	(X)	(X)	(X)	X	X	X	X	X[J]
AE/SAE assessment				X	X	X	X	X	X	X	(X)	(X)	(X)	X	X	X	X	X[J]
Solicited Local/Systemic AEs[A]							X	X	X	X	(X)	(X)	(X)	(X)[M]	X	X	X	
Conmed review			X	X	X	X	X	X	X	X	(X)	(X)	(X)	X	X	X	X	X[J]
ECG - Cardiology			X															
Treatment with AL[B]				X	X	X					(X)	(X)	(X)					
PfSPZ Vaccination or Placebo							X		X						X			
LABORATORY PROCEDURES	**Tube type[K]**	**Designated laboratory**																
Screening/safety labs																		
CBC with differential	EDTA	MRTC (CPA/UB/LPD)	0.5[L]				(0.5)[L]		0.5					0.5				
ALT, Cr	SST		0.5[L]				(0.5)[L]		0.5					0.5				
HBV, HCV, HIV testing	SST		5															
Malaria infection assays																		
Retrospective Peripheral blood smear[C,D]	EDTA	MRTC (CPA/UB/LPD)		0.5			0.5		0.5					0.5	0.5			(0.5)[D,I]
Retrospective qPCR[D]	EDTA	LMIV	0.5	0.5			0.5		0.5					0.5	0.5			(0.5)[D,I,J1]
Research assays[E]																		
Humoral assays[F]	SST	LMIV/ MRTC (CPA/UB/LPD)		5										5				
Cellular assays[G]	NaHep			5										5		10		
Transcriptional assays	PAXgene/ Nucleic Acid Stabilizer			1			1							1		1		
Parasite purification	EDTA																	(4)[J2]
Ex vivo	EDTA (pediatric)			0.5										0.5		0.5		(0.5)[J3]
Estimated Maximum Daily Total[H]			6.5	12.5	0	0	3	0	2	0	0	0	0	13.5	1	11.5	0	5.5
Estimated Maximum Study Cumulative Total			6.5	19	19	19	22	22	24	24	24	24	24	37.5	38.5	50	50	NA

A) Systemic and Local reactogencity related to PfSPZ Vaccination will be collected for 7 days after administration of PfSPZ Vaccine/Placebo.

B) AL dosing to occur **AFTER** labs collected

C) Blood smear will be run Real Time for symptomatic subjects

D) If a Blood smear (BS) is collected at an unscheduled visit due to suspected malaria, a sample for retrospective malaria qPCR should also be obtained at the same time (beginning after vaccination #1)

E) On days where multiple research assays are collected, if unable to obtain blood for all samples, collection should be prioritized in the following order: 1) Humoral, 2) Transcriptional, 3) Ex-vivo, 4) Cellular. If research blood draws are not fully completed this will not be considered a protocol deviation.

F) For humoral assays, 2-5mls can be collected based on investigators discretion.

G) For cellular assays, 5-10 mls can be collected based on investigators discretion.

H) ≤ 15ml of blood should be drawn per day in total; volume amounts per tube are estimates

I) If clinically indicated

J) Starting after vaccination #1,if if the subject has malaria symptoms AND has a positive blood smear (BS) then he/she should return within 48 hours from draw of positive BS for additional sample collection, as needed (ex-vivo, transcriptional assay, qPCR). Subjects/gaurdian may decline to return for additional blood draw (not a protocol deviation). Preference will be to obtain BS positive blood samples for the FIRST positive smear after vaccination #3, >=14 days post antimalarial treatment, new positive BS after having a negative BS >28 days.

1) qPCR to be collected at unscheduled malaria positive BS visit if sample not obtained within the last two days with the positive blood smear
2) parasite purification should be collected at unscheduled malaria positive BS visit if not collected in the last 28 days. If collected in the last 28 days, this 4ml collection can be deferred.
3) ex-vivo: to be collected at unscheduled malaria positive BS visit if sample has not been completed in the previous 2 days.

K) Tube types listed in this table may be substituted for acceptable alternatives if the PI and the leads of the immunology and/or clinical labs (as applicable) agree to the change. Any substitutions will be documented in lab logs.

L) These labs will be repeated prior to V1 if drawn 28 days or more before V1 is scheduled

M) AL clearance prior to 3rd vaccinations (Study Days 12, 13, and 14) may be deferred at PI discretion per Section 5.1. If the 2nd AL clearance is not performed, the subject will be questioned on Study Day 22 about any reactogenicity that occurred within 7 days after the 2nd PfSPZ administration.

Table 2).

Table 1. PfSPZ Vaccination Period

Clinic Visits			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Unscheduled Visit
Study Day			-73	-17	-16	-15	1	3	8	10	(12)[M]	(13)[M]	(14)[M]	22	29	32	36	NA
Days post PfSPZ Vaccination #1			-	-	-	-	0	2	7	9	11	12	13	21	28	31	35	NA
Days post last PfSPZ Vaccination			-	-	-	-	0	2	0	2	4	5	6	14	0	3	7	NA
Visit window (days)			→	-24/+10	→	→	0	+/-1	-1/+3	-1/+3	+7/-1	→	→	+/-3	+/-3	+/-1	+/-2	NA
			Screening	Enrollment and AL dosing	AL dose	AL dose	PfSPZ Vac #1	Safety f/u	PfSPZ Vac #2	Safety f/u	AL dosing	AL dose	AL dose	Pre-Vac	PfSPZ Vac #3	Safety f/u	Safety f/u	*Interim Clinical OR Positve f/u visit*
CLINICAL PROCEDURES																		
Complete medical history/physical			X															
Informed consent			X															
Malaria comprehension exam			X															
Pre-test/post-test HIV counseling			X															
Interim clinical evaluation				X	X	X	X	X	X	X	(X)	(X)	(X)	X	X	X	X	X[J]
AE/SAE assessment				X	X	X	X	X	X	X	(X)	(X)	(X)	X	X	X	X	X[J]
Solicited Local/Systemic AEs[A]							X	X	X	X	(X)	(X)	(X)	(X)[M]	X	X	X	
Conmed review			X	X	X	X	X	X	X	X	(X)	(X)	(X)	X	X	X	X	X[J]
ECG - Cardiology			X															
Treatment with AL[B]				X	X	X					(X)	(X)	(X)					
PfSPZ Vaccination or Placebo							X		X						X			
LABORATORY PROCEDURES	**Tube type[K]**	**Designated laboratory**																
Screening/safety labs																		
CBC with differential	EDTA	MRTC (CPA/UB/LPD)	0.5[L]				(0.5)[L]		0.5					0.5				
ALT, Cr	SST		0.5[L]				(0.5)[L]		0.5					0.5				
HBV, HCV, HIV testing	SST		5															
Malaria infection assays																		
Retrospective Peripheral blood smear[C,D]	EDTA	MRTC (CPA/UB/LPD)		0.5			0.5		0.5					0.5	0.5			(0.5)[D,I]
Retrospective qPCR[D]	EDTA	LMIV	0.5	0.5			0.5		0.5					0.5	0.5			(0.5)[D,I,J1]
Research assays[E]																		
Humoral assays[F]	SST	LMIV/ MRTC (CPA/UB/LPD)		5										5				
Cellular assays[G]	NaHep			5										5		10		
Transcriptional assays	PAXgene/ Nucleic Acid Stabilizer			1			1							1		1		
Parasite purification	EDTA																	(4)[J2]
Ex vivo	EDTA (pediatric)			0.5										0.5		0.5		(0.5)[J3]
Estimated Maximum Daily Total[H]			*6.5*	*12.5*	*0*	*0*	*3*	*0*	*2*	*0*	*0*	*0*	*0*	*13.5*	*1*	*11.5*	*0*	*5.5*
Estimated Maximum Study Cumulative Total			*6.5*	*19*	*19*	*19*	*22*	*22*	*24*	*24*	*24*	*24*	*24*	*37.5*	*38.5*	*50*	*50*	*NA*

A) Systemic and Local reactogencity related to PfSPZ Vaccination will be collected for 7 days after administration of PfSPZ Vaccine/Placebo.

B) AL dosing to occur **AFTER** labs collected

C) Blood smear will be run Real Time for symptomatic subjects

D) If a Blood smear (BS) is collected at an unscheduled visit due to suspected malaria, a sample for retrospective malaria qPCR should also be obtained at the same time (beginning after vaccination #1)

E) On days where multiple research assays are collected, if unable to obtain blood for all samples, collection should be prioritized in the following order: 1) Humoral, 2) Transcriptional, 3) Ex-vivo, 4) Cellular. If research blood draws are not fully completed this will not be considered a protocol deviation.

F) For humoral assays, 2-5mls can be collected based on investigators discrection.

G) For cellular assays, 5-10 mls can be collected based on investigators discrection.

H) ≤ 15ml of blood should be drawn per day in total; volume amounts per tube are estimates

I) If clinically indicated

J) Starting after vaccination #1,if if the subject has malaria symptoms AND has a positive blood smear (BS) then he/she should return within 48 hours from draw of positive BS for additional sample collection, as needed (ex-vivo, transcriptional assay, qPCR). Subjects/gaurdian may decline to return for additional blood draw (not a protocol deviation). Preference will be to obtain BS positive blood samples for the FIRST positive smear after vaccination #3, >=14 days post antimalarial treatment, new positive BS after having a negative BS >28 days.

1) qPCR to be collected at unscheduled malaria positive BS visit if sample not obtained within the last two days with the positive blood smear

2) parasite purification should be collected at unscheduled malaria positive BS visit if not collected in the last 28 days. If collected in the last 28 days, this 4ml collection can be deferred.

3) ex-vivo: to be collected at unscheduled malaria positive BS visit if sample has not been completed in the previous 2 days.

K) Tube types listed in this table may be substituted for acceptable alternatives if the PI and the leads of the immunology and/or clinical labs (as applicable) agree to the change. Any substitutions will be documented in lab logs.

L) These labs will be repeated prior to V1 if drawn 28 days or more before V1 is scheduled

M) AL clearance prior to 3rd vaccinations (Study Days 12, 13, and 14) may be deferred at PI discretion per Section 5.1. If the 2nd AL clearance is not performed, the subject will be questioned on Study Day 22 about any reactogenicity that occurred within 7 days after the 2nd PfSPZ administration.

Table 2. PfSPZ Post-vaccination malaria assessment period

Clinic and Laboratory Procedures NHMI Surveillance																
	Clinic Visits		16	17	18	19	20	21	22	23	24	25	26	27	28	Unscheduled Visit
	Study Day		43	57	71	85	99	113	127	141	155	169	183	197	211	NA
	Days post PfSPZ Cvac Vaccination #3		14	28	42	56	70	84	98	112	126	140	154	168	182	NA
	Visit window (days)		+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-5	NA
			Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u	Malaria f/u and Final Unblinding Visit	*Interim Clinical OR Positve f/u visit*
CLINICAL PROCEDURES																
Interim clinical evaluation			X	X	X	X	X	X	X	X	X	X	X	X	X	X[H]
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X[H]
Solicited malaria monitoring symptoms			X	X	X	X	X	X	X	X	X	X	X	X	X	X[H]
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X[H]
LABORATORY PROCEDURES	**Tube type[J]**	**Designated laboratory**														
Screening/safety labs																
CBC with differential	EDTA	MRTC (CPA/UB/ LPD)	0.5												0.5[G]	
ALT, Cr	SST		0.5													
Malaria infection assays																
Retrospective Peripheral blood smear[A,B]	EDTA	MRTC (CPA/UB/ LPD)	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[B,H]
Retrospective LMIV qPCR[B]	EDTA	LMIV	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[H]	0.5	(0.5)[B,H,I1]
Research assays[C]																
Humoral assays[D]	SST	LMIV/ MRTC (CPA/UB/ LPD)	5		5										5	
Cellular assays[E]	NaHep		5		5										5	
Transcriptional assays	PAXgene/ Nucleic Acid Stabilizer		1		1										1	
Parasite Purification	EDTA															(4)[I2]
Ex vivo	EDTA		0.5		0.5										0.5	(0.5)[I3]
Estimated Maximum Daily Total[F]			*13.5*	*0*	*12.5*	*0*	*1*	*0*	*1*	*0*	*1*	*0*	*1*	*0*	*13*	*5.5*
Estimated Maxium Study Cumulative Total			*63.5*	*0*	*76*	*0*	*77*	*0*	*78*	*0*	*79*	*0*	*80*	*0*	*93*	*NA*

A) Blood smear will be run Real Time in Symptomatic subjects

B) If a Blood smear (BS) is drawn at a visit due to suspected malaria, a sample for retrospective malaria qPCR should also be obtained at the same time

C) On days where multiple research assays are collected, if unable to obtain blood for all samples, collection should be prioritized in the following order: 1) Humoral, 2) Transcriptional, 3) Ex-vivo, 4) Cellular. If research blood draws are not fully completed this will not be considered a protocol deviation.

D) For humoral assays, 2-5mls can be collected based on investigators discretion.

E) For cellular assays, 5-10mls can be collected based on investigators discretion.

F) ≤ 15ml of blood should be drawn per day in total; volume amounts per tube are estimates

G) Only hemoglobin measurement is required

H) If clinically indicated

I) Starting after vaccination #1,if the subject has malaria symptoms AND has a positive blood smear (BS) then he/she should return within 48 hours from draw of positive BS for additional sample collection, as needed (ex-vivo, transcriptional assay, qPCR). Subjects/ gaurdians may refuse research blood draws without a protocol deviation. Preference will be to obtain BS positive blood samples for the FIRST positive smear after vaccination #3, >=14 days post antimalarial treatment, new positive BS after having a negative BS >28 days.

1) qPCR to be collected at unscheduled malaria positive BS visit if sample not obtained within the last two days with the positive blood smear

2) parasite purification should be collected at unscheduled malaria positive BS visit if not collected in the last 28 days. If collected in the last 28 days, this 4ml collection can be deferred.

3) ex-vivo: to be collected at unscheduled malaria positive BS visit if sample has not been completed in the previous 2 days.

J) Tube types listed in this table may be substituted for acceptable alternatives if the PI and the leads of the immunology and/or clinical labs (as applicable) agree to the change. Any substitutions will be documented in lab logs.

3. INTRODUCTION

3.1 STUDY RATIONALE

Malaria is a major global health problem, especially in sub-Saharan Africa. Progress in reducing worldwide morbidity and mortality has stalled, with the World Health Organization reporting an upward trend in the number of clinical cases from 2014 (217,000,000) to 2019 (229,000,000) (1) and no significant progress in reducing mortality during the past year (411,000 deaths in 2018, 409,000 deaths in 2019). The burden of malaria is most severe in sub-Saharan Africa, where 17 countries reported an increase in the number of cases in 2018 compared to 2015-2017 (2). Mali is one of the eleven highest burden countries in the world, with the estimated rate of cases per 1000 population exceeded in 2018 only by the rate in Burkina Faso (1). Clearly, a vaccine that could prevent infection and thereby block Pf *malaria with symptoms* and transmission would strongly support the goal of malaria elimination.

One of the most promising vaccine candidates is Sanaria® PfSPZ Vaccine, comprised of whole, radiation-attenuated *P. falciparum* (Pf) sporozoites (SPZ). Clinical trials of PfSPZ Vaccine in the US, Europe, and six African countries have shown PfSPZ Vaccine to be safe and well-tolerated in malaria-naive adults and in African adults, teenagers, children, infants and HIV+ individuals, with no difference in adverse event profiles between vaccine recipients and normal saline controls in randomized, double blind, placebo-controlled trials (3-10). In these studies, and in additional non-randomized studies, PfSPZ Vaccine has induced high levels of protection against homologous and heterologous controlled human malaria infection (CHMI) in malaria-naive and malaria exposed adults (4-6, 11-15). It has also shown modest protection against naturally transmitted Pf malaria infection over a six-month transmission season in malaria-exposed adults in four separate trials, including three conducted in Mali (Sissoko unpublished, Sirima unpublished)(9). Demonstration of vaccine efficacy (VE) in pediatric populations is limited, however. Three Phase 1 trials in Tanzania, Kenya and Equatorial Guinea and one Phase 2 trial in Kenya have been completed in infants and children ranging in age from 5 months to 17 years, and all four trials demonstrated excellent safety and tolerability, with no statistically significant difference in adverse event rates between vaccine recipients and normal saline placebo controls (Oneko submittted, Jongo unpublished)(3, 10). Data on VE in pediatric populations are now needed.

The Phase 2 trial in Kenyan infants was the first pediatric study with PfSPZ Vaccine to measure VE. The trial, called KSPZV1, showed efficacy against both parasitemia and Pf *malaria with symptoms* at 3 months in the highest dose group ($1.8x10^6$ PfSPZ Vaccine), but not at the primary 6-month endpoint, a result believed to be related to the absence of measurable cellular responses to the vaccine in these young children, all of whom were less than 1 year of age at the time of vaccination; it was hypothesized that the infant immune system is not mature enough to develop the CD8 T cell responses that underlie the durable protection induced by PfSPZ Vaccine in other age groups. More recently, a second pediatric protection study was initiated in 1-to-12-year-olds in Gabon, also a randomized, double blind, placebo-controlled trial measuring VE against naturally transmitted Pf malaria. This trial, called LaSPZV1, is still underway in Lambaréné, a region of Central Africa characterized by year-round Pf transmission and thus different from the Sahel, where transmission is highly seasonal. Results from the LaSPZV1 study are expected in the next few months. To date, no studies have been performed in pediatric populations in the Sahel.

This protocol describes the first such pediatric trial. It will be unique as the first pediatric trial in a region characterized by intense seasonal Pf transmission. It will also be the second trial looking at efficacy in pre-school and school-age children after LaSPZV1. The study is powered to show protection against first episodes of Pf *malaria with symptoms* over the six-month transmission season (primary objective), an important pediatric health need, and against first episodes of parasitemia

(secondary objective), a more stringent endpoint representing the prevention of both clinical disease and transmission. Positive results from this trial will set the stage for future large-scale Phase 3 trials. In correspondence with the US FDA, the agency has emphasized the importance of field efficacy studies in both adults and children and requires promising Phase 2 results prior to approving the Phase 3 testing plan. For this reason, the trial described herein is on the critical pathway to collecting the protection data needed for PfSPZ Vaccine licensure by the FDA.

3.2 BACKGROUND

Sanaria® PfSPZ Vaccine is comprised of aseptic, purified, cryopreserved, Pf sporozoites (PfSPZ) that are manufactured according to current Good Manufacturing Practices (cGMPs) for parenteral injection. They are diluted in phosphate buffered saline (PBS) and human serum albumin (HSA) to obtain the desired dose and concentration and are administered by direct venous inoculation (DVI) without an adjuvant. The PfSPZ are attenuated by exposure to radiation during manufacture and are unable to cause parasitemia in humans or undergo transmission to mosquitoes that might bite an individual following immunization.

3.2.1 Nonclinical Investigations of PfSPZ Vaccine

The safety and immunogenicity of PfSPZ Vaccine administered by subcutaneous (SC), intradermal, and IV routes were demonstrated in New Zealand White rabbits and mice. Three preclinical toxicology studies in rabbits given 5-7 doses of $1.35x10^5$ PfSPZ by the ID, SC and IV routes were performed. In all 3, PfSPZ Vaccine was safe and well tolerated. More details are available in the PfSPZ Vaccine Investigator's Brochure (IB).

Formal biodistribution studies were performed, which examined the distribution and persistence in mouse tissues of Pf DNA following SC, ID, or IV inoculation of a single $1.35x10^5$ PfSPZ dose of PfSPZ Vaccine. The studies showed that PfSPZ Vaccine was safe and well tolerated in mice and that there was no persistence of PfSPZ in the host tissue beyond 144 hours. The dose of sporozoites in these studies was equivalent to the highest dose administered in the first two Phase 1 clinical trials of PfSPZ Vaccine, a dose that has now been shown to be safe and well tolerated in adult and pediatric participants administered PfSPZ Vaccine by SC, ID, and/or IV routes. Details are available in the PfSPZ Vaccine IB.

Recently, a reproductive toxicology study was performed in female New Zealand White rabbits. Fifty animals were dosed intravenously via marginal ear vein with phosphate-buffered saline (PBS) containing 1% human serum albumin (HSA) and 50 with 1.8×10^6 PfSPZ of PfSPZ Vaccine with 1% HSA in PBS. Animals received 4 vaccinations: 35, 28, and 7-10 days prior to mating (study days 1, 8 and 29) and on gestation day 8. The study was interrupted prior to the planned fifth immunization due to HSA-induced anaphylaxis in vaccine and placebo recipients. However, pathological assessments showed no findings related to PfSPZ Vaccine or presence of maternally derived PfSPZ antibodies, which were developed by all vaccinees, including:

1. No redness or swelling observed at the injection site;
2. No effects on maternal body weight or food consumption;
3. No effect on maternal clinical pathology parameters (hematology, clinical chemistry, coagulation);
4. No effects on mating, fertility, or laparohysterectomy parameters, including the number of corpora lutea, implantations, live/dead fetuses, early/late resorptions, or pre- and post-implantation loss;

5. No mortality related only to PfSPZ Vaccine (2 deaths in PfSPZ Vaccine, 1 death in control, all diagnosed as anaphylaxis related to HSA administration).

These findings are consistent with the favorable safety profile obtained in clinical trials. They have been submitted to the FDA in support of a planned study of PfSPZ Vaccine in pregnant women, to be conducted this year in Mali.

3.2.2 Clinical Experience with PfSPZ Vaccine

Twenty clinical trials of PfSPZ Vaccine have been conducted or are ongoing at 4 sites in the USA, 1 site in Germany, 1 site in the Netherlands and 6 countries in Africa (Tanzania, Kenya, Mali, Burkina Faso, Gabon, Equatorial Guinea) (**Table 3**). In these trials, as of 15 March 2021, 5606 doses of PfSPZ Vaccine (over 5.2 billion PfSPZ) have been administered to 1726 subjects aged 5 months to 65 years; this includes 873 doses administered by DVI to 330 infants. In all trials, the PfSPZ have been safe and well tolerated. Details of each trial and the important findings available to date are included in the PfSPZ Vaccine IB. Safety results are further presented in the section below on risks and benefits.

Table 3. Chronological listing of trials of PfSPZ Vaccine

Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Participants**
1. NMRC/UMB CVD (NCT01001650) May 2009 *(completed)*	Phase 1, open-label, dose-escalation with CHMI in USA **(ID, SC only)**	$7.5x10^3$ SC x 4; $7.5x10^3$ ID x 4; $3x10^4$ SC x 4; $3x10^4$ ID x 4; $1.35x10^5$ SC x 4 or 6; $1.35x10^5$ ID x 4 or 6	Malaria-naïve adults: 80
2. VRC 312 (NCT01441167) Oct 2011 *(completed)*	Phase 1, open-label, dose-escalation with CHMI in USA **(IV by catheter)**	$2x10^3$ IV x 2; $7.5x10^3$ IV x 4 or 6; $3x10^5$ IV x 4 or 6; $1.35x10^5$ IV x 4 or 5	Malaria-naïve adults: 40
3. VRC 314 (NCT02015091) Dec 2013 *(completed)*	Phase 1, open-label, dose-escalation, regimen comparison with CHMI in USA **(IV by catheter or IM)**	$2.2x10^6$ IM x 4; $1.35x10^5$ IV x 4 + $4.5x10^5$ IV boost; $2.7x10^5$ IV x 3 or 4; $2.7x10^5$ IV x 2 + $4.5x10^5$ IV x 2; $9.0x10^5$ IV x 3	Malaria-naïve adults: 93
Administration in all following trials by **DVI only**			
4. Mali 1 (NCT01988636) Jan 2014 *(completed)*	Phase 1, randomized, double-blind placebo-controlled* field efficacy in Mali	$1.35x10^5$ + $2.7x10^5$; $2.7x10^5$ x 5	Malaria-exposed adults: 58

Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Participants**
5. BSPZV1 (NCT02132299) May 2014 *(completed)*	Phase 1, randomized, double-blind placebo-controlled* with CHMI (by needle and syringe) in Tanzania	$3x10^4$; then $1.35x10^5$; then $2.7x10^5$ $1.35x10^5$ x 5; $2.7x10^5$ x 5	Malaria-exposed adults: 49
6. WRAIR 2080 (NCT02215707) Jun 2014 *(completed)*	Phase 1, open-label, regimen comparison with CHMI in USA	$2.7x10^5$ x 5; $4.5x10^5$ x 3	Malaria-naïve adults: 45
7. EGSPZV1 (NCT02418962) Mar 2015 *(completed)*	Phase 1, open-label, dose-escalation in Equatorial Guinea	$1.35x10^5$; then $2.7x10^5$ $2.7x10^5$ x 3	Malaria-exposed adults: 23
8. BSPZV2 (NCT02613520) Dec 2015 *(completed)*	Phase 1 dose escalation, double-blind, randomized, placebo-controlled* with CHMI (by needle and syringe) in Tanzania	Adults, older children: $9x10^5$ x 3; then $1.8x10^6$ x 3 Younger children, infants: $4.5 x 10^5$ x 3; then $9x10^6$ x 3	Malaria-exposed adults: 12 children: 36 infants: 15
9. Mali 2 (NCT02627456) Jan 2016 *(completed)*	Phase 1 dose escalation with CHMI followed by Phase 2 randomized, double-blind, placebo-controlled* field efficacy in Mali	Ph 1: $4.5x10^5$ x 1; then $9x10^5$ x 1; then $1.8x10^6$ x 3 Ph 2: $1.8x10^6$ x 3	Malaria-exposed adults: 100
10. Burkina Faso 1 (NCT02663700) Apr 2016 *(completed)*	Phase 1 dose escalation followed by Phase 2, randomized, double-blind placebo-controlled* field efficacy in Burkina Faso	Ph 1: $4.5x10^5$ x 2; then $9x10^5$ x 2; then $1.8x10^6$ x 2; then $2.7x10^6$ x 2 Ph 2: $2.7x10^6$ x 3	Malaria-exposed adults: 32
11. Warfighter 2 (NCT02601716) Apr 2016 *(completed)*	Phase 2, open-label, regimen comparison with CHMI in USA	$4.5x10^5$ x 5 (Days 1, 3, 5, 7 and week 16); or $9x10^5$ x 3 (Weeks 1, 9, 17); or $1.8x10^6$ x 3 (Weeks 1, 9, 17); or $2.7x10^6$ x 1 + $9x10^5$ x 2 (Weeks 1, 9, 17)	Malaria-naive adults: 60

Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Participants**
12. KSZPV1 (NCT02687373) Jul 2016 *(completed)*	Phase 1 dose escalation followed by Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Kenya	Ph 1 - Older children: $4.5x10^5$ x 1; then $9x10^5$ x 2; then $1.8x10^6$ x 2 Ph 1 - Younger children, infants: $1.35x10^5$ x 1; then $2.7x10^5$ x 1; then $4.5x10^5$ x 1; then $9x10^5$ x 2; then $1.8x10^6$ x 2, all Ph 2 - Infants: $4.5x10^5$, $9x10^5$, or $1.8x10^6$, all x 3	Malaria-exposed children: 100 infants: 401
13. MAVACHE (NCT02704533) Sep 2016 *(completed)*	Phase 1 dose escalation, regimen-condensation and dose number reduction with CHMI in Germany	$9x10^5$ x 3 (Days 1, 8, 29); then $1.8x10^6$ x 2 (Days 1, 8); then $2.7x10^6$ x 2 (Days 1, 8)	Malaria-naive adults: 42
14. EGSPZV2 (NCT02859350) Nov 2016 *(completed)*	Phase 1 dose escalation, randomized double-blind, placebo-controlled* with head-to-head PfSPZ Vaccine and PfSPZ-CVac comparison in Equatorial Guinea	Adults (PfSPZ Vaccine): $2.7x10^6$ x 3 Adults (PfSPZ-CVac): $1x10^5$ x 3 Children, infants (PfSPZ Vaccine): $1.8x10^6$ x 3	Malaria-exposed adults: 52** children: 36 infants: 15
15. BSPZV3a (NCT03420053) *(completed)*	Phase 1 dose escalation, randomized double-blind, placebo-controlled* with CHMI in Tanzania	$4.5x10^5$ x 5 (Days 1, 3, 5, 7 and 29); or $9x10^5$ x 5 (Days 1, 3, 5, 7 and 29)	Malaria-exposed HIV- and HIV+ adults: 21
16. MSPZV3 (NCT03510481) *(completed)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Mali	$9x10^5$ x 3 (Days 1, 8 and 29); or $9x10^5$ x 3 (Weeks 1, 9, 17)	Malaria-exposed adults: 210
17. LaSPZV1 (NCT03521973) *(ongoing)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Gabon	$9x10^5$ x 3 (Days 1, 8 and 29)	Malaria-exposed children: 200
18. EGSPZV3 (NCT03590340) *(ongoing)*	Phase 1 double-blind, randomized, placebo-controlled* with CHMI in Equatorial Guinea	$9x10^5$ x 3 (Days 1, 8 and 29); or $9x10^5$ x 5 (Days 1, 3, 5, 7 and 29); or $9x10^5$ x 5 (Days 1, 3, 5, 7 and Week 17); or $9x10^5$ x 4 (Days 1, 3, 5, 7)	Malaria-exposed adults: 104
19. MSPZV4 (NCT03510481) *(ongoing)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Mali	$9x10^5$ x 3 (Days 1, 8 and 29); or $1.8x10^6$ x 3 (Days 1, 8 and 29)	Malaria-exposed women of child-bearing potential: 300

Study Identifier (Clinicaltrials.gov) Start Date	**Study Design Summary**	**PfSPZ Vaccination Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Participants**
*The placebo control used in all trials is normal saline; ** 20/52 adult volunteers in EGSPZV2 received PfSPZ-CVac.			
In these trials, dosing schedules have evaluated various intervals between vaccinations ranging from 4 to 16 weeks.			
This table omits one trial of a genetically attenuated sporozoite vaccine (PfSPZ-GA1) conducted in the Netherlands that used PfSPZ Vaccine as a comparator (total number of trials is therefore 20).			

Pediatric Trials: The studies that are most relevant to the current trial are three studies that have been completed in African children and infants (#8, #12, #14 in **Table 3**), one of which, a study of Kenyan infants, was divided into a Phase 1 age de-escalation / dose-escalation trial (Part 1) and a Phase 2 field efficacy trial (Part 2). The first pediatric study was conducted in Tanzania, an age de-escalation / dose-escalation first-in-children study for safety and tolerability (#8 in **Table 3**). The vaccine was safe and well tolerated in all age groups (3). A three-dose regimen of $9.0x10^5$ PfSPZ administered at 8-week intervals was tested in all five age groups (adults, 11-17-year-olds, 6-10-year-olds, 1-5-year-olds and infants) allowing a comparison of immune responses across age groups. Antibody responses to PfCSP in the Tanzanian adults were ~10 times lower than the antibody responses to the same immunization regimen in U.S. adults. However, the antibody responses to PfCSP were ~10 times higher in infants than in the Tanzanian adults and comparable to the antibody responses in US adults, while older children showed intermediate responses (**Figure 3**). Infants showed no T cell responses, however, while better responses were recorded in 1-5-year-olds and 6-10-year-olds **(Figure 4**). Because the durable protection induced by PfSPZ Vaccine is likely mediated by T cells, it was surmised that the vaccine might not be protective in infants. On the other hand, the strong antibody responses indicated that there might be protection, particularly soon after immunization when antibody responses remained high.

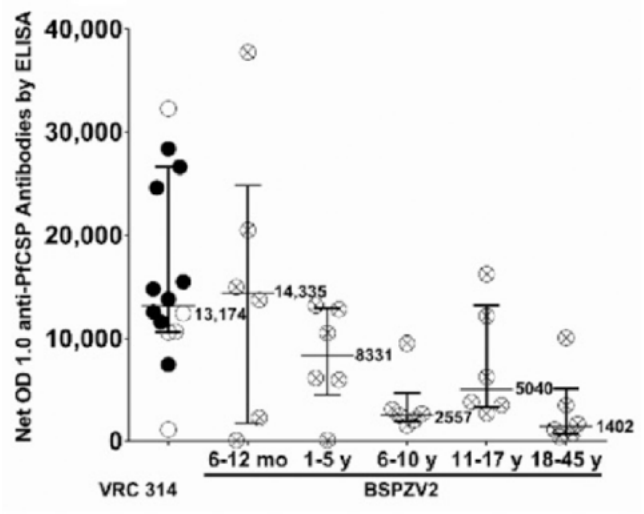
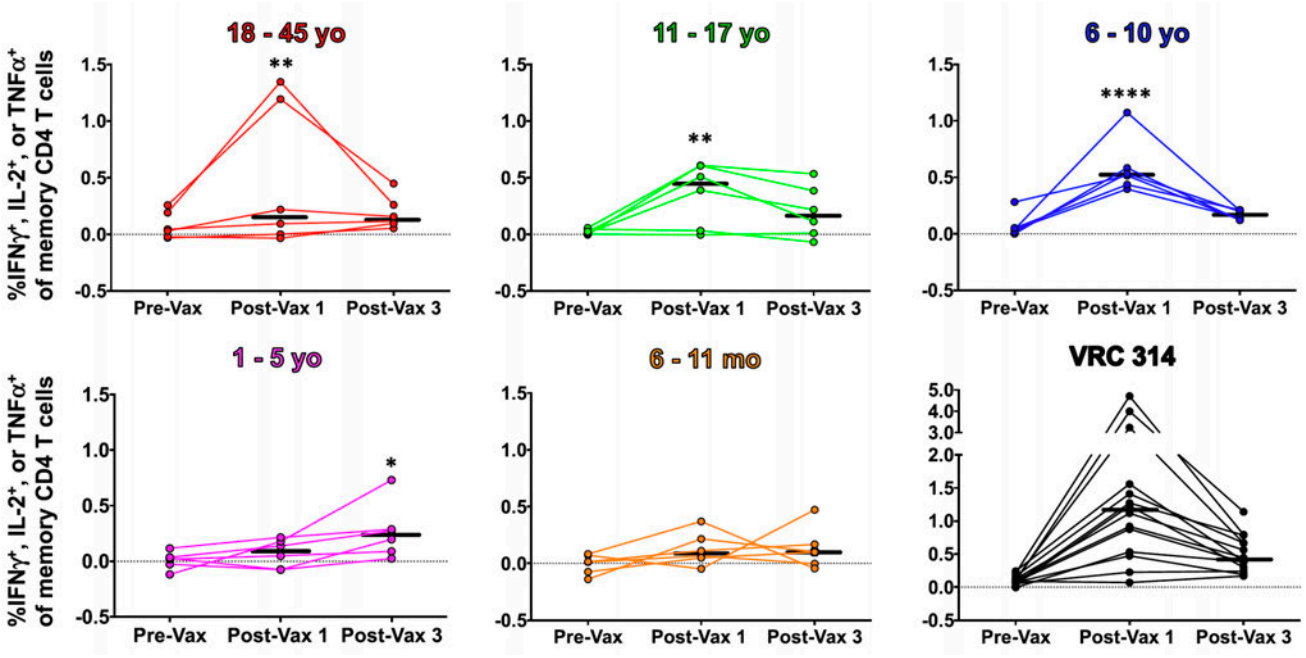


Figure 3. Difference between post-immunization and pre-immunization antibody results in BSPZV2 trial in Tanzania by age group.

All individuals were administered 3 doses of $9.0x10^5$ PfSPZ at 8-week intervals. Results were obtained by subtracting pre-immune values from those 2 weeks after the third dose. VRC314 was conducted in US adults with the same dosage regimen; filled-in circles indicate protected volunteers and empty circles the unprotected volunteers. Medians with interquartile ranges are shown. (3)

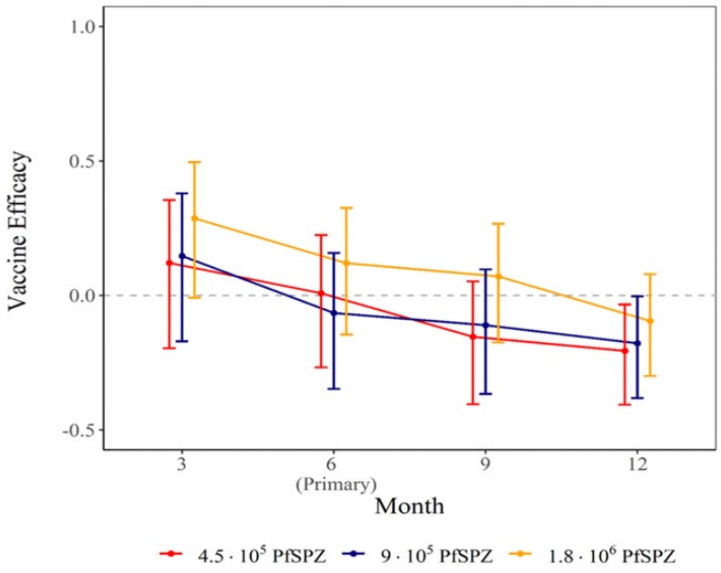
Figure 4. PfSPZ-specific memory CD4 T-cell responses pre- and post-vaccination.

Percent of memory CD4 T cells in the blood expressing interferon gamma (IFN-γ) interleukin 2 (IL-2) or tumor necrosis factor alpha (TNF-α) at pre-immunization or 2 weeks after the first and third doses of PfSPZ Vaccine ($9.0x10^5$). Results are the percentage of cytokine-producing cells after incubation with PfSPZ minus the percentage of cytokine-producing cells after incubation with vaccine diluent (medium with 1% human serum albumin). Bars indicate median values within each group. Differences within each age group between pre- and postvaccination groups were assessed by two-way ANOVA with Dunnett's correction for multiple comparisons. *P < 0.05, **P < 0.01, ****P < 0.0001. Previously measured results from clinical trial VRC 314 conducted in the US with the same dosage regimen and the same assay conducted in the same laboratory as for the BSPZV2 trial assays are shown as a comparison. (3)



The second pediatric trial was initiated six months later in western Kenya (#12 in **Table 3**) and addressed this hypothesis. After an initial age de-escalation / dose-escalation Phase 1 pilot study demonstrating that the vaccine was safe in all ages tested (10), a larger Phase 2 trial was performed in which more than 300 infants age 5-11 months were randomized to receive 3 doses of normal saline placebo or $4.5x10^5$, $9.0x10^5$, or $1.8x10^6$ PfSPZ of PfSPZ Vaccine, which was administered weeks 1, 9 and 17. Again, the vaccinations were safe, with no differences between vaccinees and normal saline placebo recipients in the rate or severity of solicited adverse events, unsolicited adverse events or laboratory abnormalities. The study subjects were followed over six months for incident malaria, and did not show statistically significant protection compared to controls over this period, although protection against both Pf *malaria with symptoms* and parasitemia was significant during the first 90 days after immunization in the highest dose group (Oneko M and Steinhardt L, submitted). The early protection likely reflected vigorous antibody responses to the immunization as measured by PfCSP ELISA. It is surmised that as the antibody response faded, so did protection (**Figure 5**). This hypothesis was supported by the strong correlation between antibody responses to PfCSP and protection in this trial (see section on "Correlates of Protection" below). A more aggressive immunization regimen may be needed to induce T cell responses in infants that provide long-term protection.

Figure 5. Vaccine efficacy at 3, 6, 9, and 12 months for the three vaccine groups (Oneko and Steinhardt, unpublished data)



A third pediatric study, EGSPV2, was conducted in Equatorial Guinea using a similar design to the Tanzanian study (addressing safety and immunogenicity, but not efficacy in children), and showed PfSPZ Vaccine to be safe and well tolerated in infants and children (same age groups as BSPZV2) (#14 in **Table 3**). Immunogenicity data from this trial are pending (Jongo S, unpublished data).

A fourth pediatric study is now underway in 1-12-year-old Gabonese children, using a Day 1, 8 and 29 regimen ($9x10^5$ PfSPZ/dose), measuring VE against naturally transmitted malaria (#17 in **Table 3**). The results of this study are expected in the next couple of months. The blinded data (combining vaccinees and placebo recipients) do not indicate any concerning safety signal.

In summary, trials of PfSPZ Vaccine in four African countries indicate that the vaccine is safe and well tolerated in all pediatric age groups. This includes doses up to $1.8x10^6$ PfSPZ in infants. As the dose planned for 6-to-10-year-olds in the current study is half that dose ($9.0x10^5$) and will be studied in 6-10-year-olds, we do not anticipate any safety issues. Additional information on safety, including specific safety data for pediatric age groups as well as a meta-analysis of the pediatric trials, is presented below in the section on risks and benefits.

Vaccine Efficacy: PfSPZ Vaccine administered intravenously at adequate doses has produced 80-100% protection against both homologous and heterologous controlled human malaria infection (CHMI) in malaria-naive adults in multiple studies (11-13, 15). High level protection has also been induced in malaria-exposed African adults; in a study in Tanzania (the BSPZV2 clinical trial), 6/6 (100%) adult volunteers undergoing homologous CHMI 3 to 10 weeks after receiving 3 doses of $9.0x10^5$ PfSPZ of PfSPZ Vaccine administered at 8-week intervals were protected (4) and in Mali, 30/30 (100%) adults receiving 3 doses of $1.8x10^6$ PfSPZ of PfSPZ Vaccine also at 8-week intervals were protected (Sissoko M and Healy S, unpublished). VE extended to protection against naturally transmitted malaria in the field: in three studies of PfSPZ Vaccine in Mali, immunization provided 51-57% protection by time-to-event analysis (VE = 1 minus the hazard ratio) and 24-35% protection by relative risk analysis (VE = 1 minus the risk ratio) against naturally transmitted malaria during a period of six months (Sissoko M and Healy S, unpublished)(9). In a similar trial in Burkina Faso, immunization provided 47% protection by time-to-event analysis and 38% protection by relative risk

analysis against naturally transmitted malaria during a period of six months (Sirima S and Lauren M, unpublished). In the same trial, VE was 46% at 18 months by time to event analysis (p=0.018). In all these trials, VE in the field has been measured against malaria *infection*, not clinical disease, meaning that the protected research subjects remained free of parasitemia as detected by thick blood smear (TBS) over six months. These results indicated that PfSPZ Vaccine should be a powerful intervention for malaria control and elimination in Africa.

Dose selection: In the US/EU and in some African studies, $9.0x10^5$ PfSPZ appeared to be the optimal dose, and higher doses were less protective. For example, in the second CHMI assessment of VE in Tanzania (BSPZV2), when the dose was increase from $9.0x10^5$ to $1.8x10^6$ PfSPZ, VE fell from 100% to 33% (4); in the first CHMI trial in Equatorial Guinea (EGSPZV2), less than 50% of adults were protected after receiving three injections at 8-week intervals with a still higher dose, $2.7x10^6$ PfSPZ of PfSPZ Vaccine (6). On the other hand, the $1.8x10^6$ PfSPZ dose achieved 100% protection in Mali, performed in a location where naturally acquired immunity (NAI) was strongly developed (Sissoko M and Healy S, unpublished). In this case, NAI may have reduced the effective dose by neutralizing a proportion of the injected PfSPZ, such that the results reflected those of lower doses in other African countries where NAI is less fully developed. This finding was supported by the results of a study in Malian women of child-bearing potential (WOCBP, MLSPZV4): both $9.0x10^5$ and $1.8x10^6$ PfSPZ were tested head-to-head for protection against naturally transmitted Pf malaria, and the higher dose resulted in stronger VE (57% vs 41%), although the difference in VE between the two groups was not statistically significant (Sissoko M and Healy S, unpublished). Thus, it may be that higher doses are needed in those with heavy prior malaria exposure than for malaria-naïve individuals, for children living in endemic areas who have experienced less malaria exposure than their parents, or with adults with only limited exposure. At this point, our best estimate is that $9.0x10^5$ PfSPZ will be an appropriate dose to assess in pre-school and school-age children in the current study.

Vaccine intervals: Recent data indicate that accelerated vaccination schedules provide better VE, particularly when two or more priming doses are administered in quick succession. Until this effect was uncovered, earlier studies had assessed longer vaccination schedules, where 3 to 5 doses were administered over 16 to 20 weeks, with relatively even spacing. In the Warfighter 2 trial in the US, however, a dose of $4.5x10^5$ PfSPZ of PfSPZ Vaccine was administered on Days 1, 3, 5 and 7, with a boost at week 17. This condensed priming regimen produced an excellent humoral immune response and 40% VE against a heterologous strain of malaria (heterologous = different from the vaccine strain) at three months (14). In this case, CHMI was administered by the bite of mosquitoes infected with a strain of malaria from Brazil, while the vaccine strain, NF54, originates from West Africa (16, 17). A heterologous CHMI at 3 months is highly stringent. Yet the 40% protection achieved was twice the VE achieved against CHMI by three other regimens studied in the Warfighter 2 trial, each of which followed a traditional week 1, 9 and 17 immunization schedule and with one of these regimens utilizing three doses of $9.x10^5$ PfSPZ, the dose considered optimal in malaria-naive adults. Likewise, in the MAVACHE study in Germany, $9.0x10^5$ PfSPZ administered on Days 1, 8 and 29 (weeks 1, 2 and 4) protected 14/17 (78.8%[1]) of research subjects against a homologous strain of malaria (homologous = the same strain as the vaccine) at 3 and 10 weeks and 10/12 (83.3%) of research subjects against a heterologous strain of malaria at 3 and 10 weeks (Mordmüller B, unpublished). This demonstration of cross-strain protection indicated that the condensed regimen may be effective against the diverse parasites transmitted in nature. In MLSPZV3 trial in Mali, condensed and traditional 3 dose regimens were compared head-to-head, and VE trended higher (without a statistically significant difference) following the 3-dose condensed regimen from MAVACHE (weeks 1, 2 and 4) than a

[1] Only 5/6 controls became positive

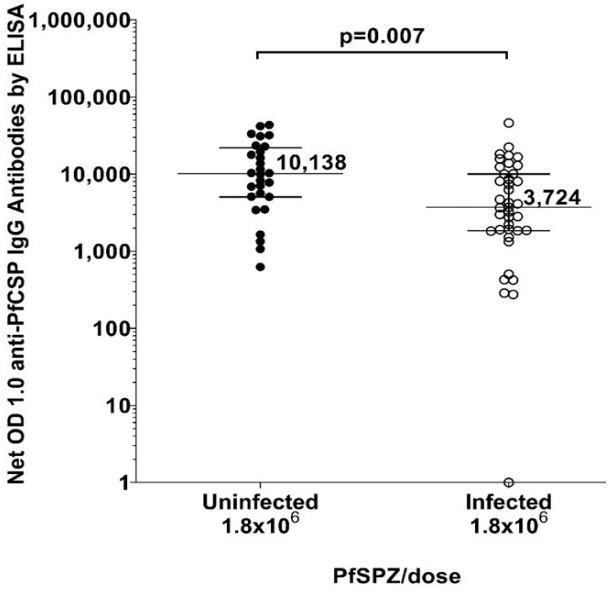
widely-spaced interval regimen (weeks 1, 7 and 15) (22% vs.14%[2]) (Sissoko M and Healy S, unpublished).

The potency of condensed regimens may relate to the multi-dose priming series – Days 1, 3, 5 and 7 in Warfighter 2, and Days 1 and 8 in the MAVACHE, MLSPZV3 and MLSPZV4 trials. It has been hypothesized that two or more doses in succession may better recruit CD8+ T cells than more widely spaced doses. Another advantage is that condensed regimens improve the ease and practicality of immunization. Completing primary immunization in four weeks will benefit travelers, pregnant women and others at risk of malaria where rapid induction of immunity is an advantage. After testing over 16 different regimens, Sanaria (the sponsor of PfSPZ Vaccine) has down-selected the 1, 8, and 29 day condensed regimen for testing in the planned Phase 3 program leading to licensure.

Correlates of protection: The level of antibodies binding to the main surface protein of PfSPZ, the Pf circumsporozoite protein (PfCSP), as measured by ELISA, or to whole PfSPZ, have correlated with protection in both malaria-naïve and malaria exposed individuals (Erreur ! Source du renvoi introuvable.) (Oneko submitted, Sirima submitted)(9, 12, 15). The availability of these standardized assays and their association with protection suggest that it may be possible to develop a marker of "vaccine take" as a surrogate for the tissue (liver) resident cellular immune mechanisms thought to underlie protection induced by whole PfSPZ-based vaccines. Data collected to date, however, show a large degree of overlap between the PfCSP antibody levels of protected and non-protected subjects. Although the correlate holds true at the population level, there is no clear cut-off for individuals that indicates protective immunity. Measuring PfCSP antibodies by ELISA will be an important objective in the current trial.

Figure 6. Anti-CSP responses in vaccinees in the KSPZV1 clinical trial (Oneko, unpublished).

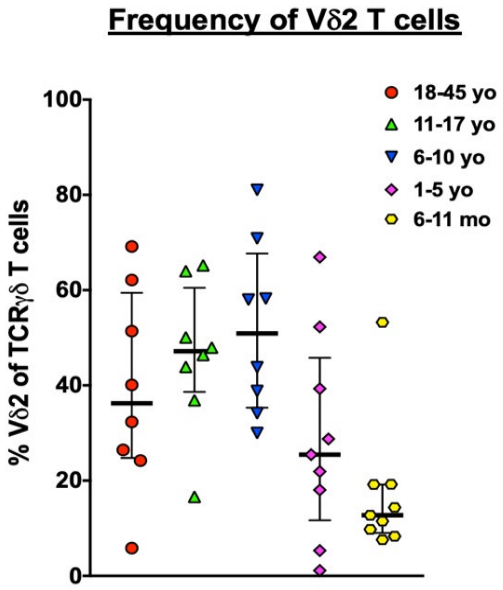
Median and interquartile range of net OD 1.0 for IgG antibodies to PfCSP two weeks after the last dose of $1.8x10^6$ PfSPZ Vaccine efficacy at 3,in participants who were uninfected (protected) and infected during 6, 9 and 12 months follow up. P value calculated by Wilcoxon-Mann-Whitney test for the three vaccine groups (Oneko and Steinhardt, unpublished data).



[2] VE in the MLSPZV3 trial was not statistically significant – the only trial among 4 similar trials conducted in the Sahel where this was the case. The poor result likely resulted from (1) failure to clear parasitemia prior to the first dose and (2) initiating the trial during the rainy season when transmission had already begun.

The underlying mechanism of protection is thought to be liver resident CD8 T cells that recognize liver-stage Pf antigens expressed on the surface of infected hepatocytes. However, it has not been possible to show a correlation between CD8 T cells responses and protection, likely because peripheral CD8 T cells populations do not reflect the tissue resident populations. However, the frequency of the Vδ2 subset of γδ T cells measured post immunization has correlated with protection in field studies and is thought to be critical to the induction phase of protective immunity (18). Interestingly, the infants studied in the BSPZV2 trial in Tanzania were assessed for Vδ2 γδ T cells in samples taken prior to immunization, and these cells were absent, as shown in **Figure 7**. This may explain why the infants in both the BSZPV2 trial in Tanzania and in the larger KSPZV1 trial in Kenya did not develop measurable CD4 or CD8 T cell responses following vaccination. Indeed, cellular immunity is now being analyzed in the Kenyan infant population, and no infants have shown measurable peripheral T cell responses (Oneko, submitted).

Figure 7. Frequency of Vδ2 γδ T cells prior to immunization in the BSPZV2 trial in Tanzania (Oneko, submitted).



Long-term development plan: The long-term objectives for PfSPZ Vaccine in malaria-exposed populations are 1) seasonal malaria prophylaxis such as will be tested in the current trial; 2) protection of pregnant women; and 3) deployment for use in mass vaccination programs (MVPs), in which entire populations in defined geographic areas will be immunized, excluding infants (based on the minimal protection seen in the Kenya study). The strategy for malaria elimination campaigns will be to combine mass drug administration (MDA) with MVPs; MDA will clear existing parasitemia and the MVP will prevent reinfection. This approach has the potential to halt malaria transmission and eliminate malaria from the target population.

The current trial is a key step on the pathway to licensing PfSPZ Vaccine for the planned indications. It will measure vaccine safety and VE in children using a randomized, double-blind, placebo-controlled design, generating critical data to support the Phase 3 program and eventual licensure in the US, EU, Mali and other malaria-endemic countries.

3.3 RISK/BENEFIT ASSESSMENT

3.3.1 Known Potential Risks

Venipuncture

Risks occasionally associated with venipuncture including the needle stick associated with DVI include pain, bruising, swelling, induration, erythema and bleeding at the site of venipuncture. Lightheadedness, and rarely, syncope can also occur. A possible but rare side effect is infection at the venipuncture site, which, if not treated promptly, could lead to more generalized symptoms such as limitation of arm movement, lymphadenopathy, fever, chills, headache, malaise, myalgia and joint pain.

The side effects associated with venipuncture are generally mild and self-limiting but will be monitored if they occur and are clinically concerning.

When children undergo needle stick, they may cry or struggle. Gentle restraint a child during venipuncture may be required and can add to the discomfort, both physical and emotional, experienced by the child.

The total amount of blood collected with venipuncture for blood drawing is within the American Association of Blood Banks recommendations and the current NIH guidelines, and will not compromise these otherwise healthy subjects (19). Any minor bruising, local tenderness or pre-syncopal symptoms, or rarely, infection associated with venepuncture, will be documented as AEs.

DVI Immunizations (either PfSPZ Vaccine or Normal Saline)

Possible local site reactions resulting from the injection of 0.5 mL of PBS with HSA/PfSPZ Vaccine or normal saline, in addition to those listed above relating to the needle stick, include additional pain, bruising, swelling, induration, erythema and bleeding should the vein be missed or should some of the injectate extravasate during injection. When the injectate is placed into the lumen of the vein as intended, local reactions are unlikely because the injectate is disbursed by blood flow.

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

PfSPZ Vaccine

As described in the background section, PfSPZ Vaccine has been exceptionally safe and well tolerated in malaria-naïve adults in the USA and Europe, and in malaria-exposed adults, children and infants in Africa. As of 15 March 2021, 5606 doses of PfSPZ Vaccine (over 5.2 billion PfSPZ) have been safely injected into 1726 subjects aged 5 months to 65 years in Tanzania, Kenya, Mali, Burkina Faso, Gabon, Equatorial Guinea, Germany, the Netherlands and the US, including 873 doses administered by DVI to 330 infants.

An early safety concern with PfSPZ Vaccine was breakthrough malaria infections due to inadequate attenuation. However, there have been no observed breakthroughs following injection with over 5,000 doses of PfSPZ Vaccine administered to date. A second concern had been local side effects at the injection site, given the reactogenicity of mosquito bites and the possibility that sporozoites may contribute. Interestingly, there appears to be little or no local reactogenicity associated with PfSPZ injection: in trials of attenuated sporozoite immunization by mosquito bite, the papules, edema, erythema and pruritus induced were equivalent between true immunization using infected mosquitoes and mock immunization using non-infected mosquitoes, indicating that mosquito saliva must be the cause (20). Additional information comes from trials of PfSPZ Vaccine and PfSPZ Challenge injected

ID and SC. These routes of administration leave a depot of PfSPZ in or under the skin, but do not cause local inflammation at the injection site, even after repeated dosing (12). The manufacturing process at Sanaria removes nearly all mosquito components, explaining the apparent absence of reactogenicity.

The third concern has been that PfSPZ Vaccine might cause systemic reactogenicity, as commonly occurs with vaccines. However, even large numbers of PfSPZ (the equivalent of perhaps thousands of infectious mosquito bites) have been remarkably well tolerated. There have been no clearly documented immediate type hypersensitivity reactions. Doses as high as $2.7x10^6$ PfSPZ have been given by DVI and as high as $2.2x10^6$ PfSPZ have been given intramuscularly (IM) without apparent side effects. In all trials so far, the majority of vaccine recipients reported no or mild systemic AEs, with only a few classified as moderate in severity. Fever has only rarely been reported as a solicited AE and the few reports to date were generally attributed to other causes.

An example of AE profiles in a relevant pediatric population is provided by the BSPZV2 trial in Tanzania, the first pediatric trial of PfSPZ Vaccine. There were no systemic AEs recorded during the seven days after immunization in either the 1-5-year-olds or 6-10-year-olds (**Table 4**). There were 6 Grade 2 and no Grade 3 laboratory abnormalities recorded post vaccination; 5/6 laboratory abnormalities occurred in the placebo group (**Table 5**).

Table 4. Solicited adverse events recorded days 0-7 post-all immunizations.
Participants received three doses weeks 1, 9, and 17 of $4.5.x10^5$, $9.0x10^5$ or $1.8x10^6$ PfSPZ of PfSPZ Vaccine.

Group 3 (6-10 years)	**$9x10^5$ (N=6)**	**$1.8x10^6$ (N=6)**	**Placebo (N=6)**
Arthralgia	0	0	0
Chills	0	0	0
Fatigue	0	0	0
Headache	0	0	0
Malaise	0	0	0
Myalgia	0	0	0
Rash, urticaria, pruritus, edema	0	0	0
Subjective fever	0	0	0
Temperature (°C)	0	0	0
Group 4 (1-5 years)	**$4.5x10^5$ (N=6)**	**$9x10^5$ (N=6)**	**Placebo (N=6)**
Drowsiness	0	0	0
Elevated Temperature	0	0	0
Fever /history of fever	0	0	0
Inability/refusal to eat or drink	0	0	0
Irritability/fussiness	0	0	0

Table 5. Total number (percent) of Grade 2 laboratory abnormalities by pediatric age group in BSPZV2 trial in Bagamoyo, Tanzania.
There were no Grade 3 abnormalities in these age groups.

Lab parameter	Group 3 (6-10 years)			Group 4 (1-5 years)		
	$9x10^5$ (N=6)	$1.8x10^6$ (N=6)	Placebo (N=6)	$4.5x10^5$ (N=6)	$9x10^5$ (N=6)	Placebo (N=6)
Leukopenia	0	0	0	0	0	0
Neutropenia	0	0	0	0	0	0
Lymphopenia	0	0	0	0	0	3 (50.0)
Eosinophilia	0	0	0	0	0	0
Decreased hemoglobin	0	0	1 (16.7)	0	0	0
Thrombocytopenia	0	0	0	1 (16.7)	0	0
Elevated creatinine	0	0	0	0	0	0
Elevated total bilirubin	0	0	0	0	0	0
Elevated ALT	0	0	1 (16.7)	0	0	0
Elevated AST	0	0	0	0	0	0

In the Kenya study (KSPZV1), Part 1 included children in the age range of the current trial (Part 2 included only infants). Although the rates of AEs were higher than in BSPZV2, there were no differences between vaccinees and normal saline placebo controls in any age groups, including the age groups comparable to the current study in Mali (**Table 6**). None of the AEs were Grade 3.

Table 6. Total number (percent) of adverse events by pediatric age group in KSPZV1 trial in Kenya

		Group 1 (5-9 years)					Group 2 (1-4 years)						
Symptom	Grade	$4.5x10^5$	$9x10^5$	$1.8x10^6$	All Vaccine	Placebo	$1.35x10^5$	$2.7x10^5$	$4.5x10^5$	$9x10^5$	$1.8x10^6$	All Vaccine	Placebo
		N=8	N=8	N=8	N=24	N=12	N=10	N=9	N=8	N=9	N=8	N=44	N=20
Any Systemic AE	Mild	1 (12.5)	1 (12.5)	2 (25.0)	4 (16.7)	4 (33.3)	3 (30.0)	0	0	0	2 (25.0)	5 (11.4)	3 (15.0)
	Mod	0	0	1 (12.5)	1 (4.2)	1 (8.3)	1 (10.0)	0	0	1 (11.1)	0	2 (4.5)	1 (5.0)
	Sev	0	0	0	0	0	0	0	0	0	0	0	0
Allergic rash /urticaria/ generalized pruritus*	Mild	N/A*	0	1 (12.5)	1 (6.3)	1 (12.5)	2 (20.0)	0	0	0	0	2 (4.5)	1 (5.0)
	Mod	N/A*	0	0	0	0	0	0	0	0	0	0	0
	Sev	N/A*	0	0	0	0	0	0	0	0	0	0	0
Drowsiness	Mild	0	0	2 (25.0)	2 (8.3)	1 (8.3)	0	0	0	0	0	0	2 (10.0)
	Mod	0	0	0	0	0	0	0	0	0	0	0	0
	Sev	0	0	0	0	0	0	0	0	0	0	0	0
Irritability /fussiness	Mild	0	0	0	0	0	0	0	0	0	0	0	1 (5.0)
	Mod	0	0	0	0	0	0	0	0	0	0	0	0
	Sev	0	0	0	0	0	0	0	0	0	0	0	0
Inability to eat	Mild	0	0	0	0	0	0	0	0	0	0	0	0
	Mod	0	0	1 (12.5)	1 (4.2)	1 (8.3)	1 (10.0)	0	0	0	0	1 (2.3)	1 (5.0)
	Sev	0	0	0	0	0	0	0	0	0	0	0	0
Fever (axillary)	Mild	1 (12.5)	1 (12.5)	1 (12.5)	3 (12.5)	4 (33.3)	2 (20.0)	0	0	0	2 (25.0)	4 (9.1)	2 (10.0)
	Mod	0	0	0	0	0	0	0	0	1 (11.1)	0	1 (2.3)	0
	Sev	0	0	0	0	0	0	0	0	0	0	0	0

Mild (Grade 1); Mod = moderate (Grade 2); Sev = severe (Grade 3); *Allergic rash/urticaria/generalized pruritus were not collected for 5–9-year-olds receiving $4.5x10^5$ or placebo.

The same held true with laboratory abnormalities – similar rates in vaccinees and controls, and no Grade 3 abnormalities (**Table 7**).

Table 7. Day 8 post-immunization laboratory abnormalities of any grade by dose group, number of subjects (percent).
All age groups (6 months to 9 years) combined.

Lab Parameter	$1.35x10^5$ (N=18)	$2.7x10^5$ (N=18)	$4.5x10^5$ (N=24)	$9x10^5$ (N=27)	$1.8x10^6$ (N=24)	All Vaccine (N=111)	Placebo (N=53)
Hemoglobin	1 (5.6)	5 (27.8)	6 (25.0)	6 (22.2)	5 (20.8)	23 (20.7)	15 (28.3)
White Blood Cells	0	0	0	3 (11.1)	0	3 (2.7)	3 (5.7)
Platelets	0	0	1 (4.2)	1 (3.7)	0	2 (1.8)	2 (3.8)
Neutrophil Count	1 (5.6)	3 (16.7)	1 (4.2)	3 (11.1)	0	8 (7.2)	4 (7.5)
ALT	1 (5.6)	0	0	0	0	1 (0.9)	2 (3.8)
Creatinine	0	0	1 (4.2)	0	1 (4.2)	2 (1.8)	1 (1.9)

One subject in the $2.7x10^5$ group terminated early before follow-up labs were collected. Abnormal lab values were Grade 1 or Grade 2, none were Grade 3.

The largest study of children in the age range planned for the current study is the LaSPZV1 trial. Although the data are still blinded, the composite data combining vaccinees and controls do not indicate any safety signals. **Table 8** and **Table 9** provide data on solicited adverse events and laboratory abnormalities, respectively.

Table 8. Solicited adverse events recorded days 0-7 post-all immunizations.
Participants received three doses weeks 1, 2 and 5 of $9.0x10^5$ PfSPZ of PfSPZ Vaccine. Data from vaccine and normal saline placebo groups combined (trial remains blinded). Data are the number of participants experiencing the AEs.

AE	Severity	1-2 years (n=36)	3-6 years (n=101)	7-12 years (n=63)
Fever (objective)	Mild (38-38.4ºC)	3	1	3
	Moderate (38.5-38.9)	1	4	1
	Severe (≥39.0ºC)	1	1	0
poor feeding	Mild	2	1	not applicable
fever (subjective)	Mild	not applicable		1
	Moderate			2
chills	Mild			1
fatigue	Mild			3
	Moderate			3
headache	Mild			8
	Moderate			2
arthralgias	Mild			0
	Moderate			1
myalgias	Mild			0
	Moderate			1

Subjective fever: parent's impression that child had fever within past 24 hours.

Children age 1-6 were scored with regard to inability to eat, drowsiness and irritability /fussiness; there were no instances of drowsiness or irritability/fussiness.

Children age 7-12 were questioned with respect to solicited symptoms. There were no recorded instances of malaise. In all three age groups, there were no findings suggestive of an allergic reaction, including rash, urticaria, pruritis, or edema.

Table 9. Laboratory abnormalities Grade 2 or higher by age group, days 1 to 28 post-immunization.

Tests were scheduled 1 and 7 days after each vaccine dose and prior to V3. The table also includes any unscheduled lab tests. Data from vaccine and normal saline placebo groups combined (trial remains blinded). Data are the number of participants experiencing the abnormalities and also the number of abnormalities experienced (no child had more than one lab abnormality of a given type).

Laboratory abnormality	Severity Grade	1-2 years (n=36)	3-6 years (n=101)	7-12 years (n=63)
ALT elevation	Grade 2 or higher	0	0	0
Creatinine elevation	Grade 2	0	0	1
	Grade 3 or higher	0	0	0
Hemoglobin decrease	Grade 2 or higher	0	0	0
WBC decrease	Grade 2 or higher	0	0	0
Platelet count decrease*	Grade 2	0	0	1
	Grade 3	1	2	0
	Grade 4	0	0	1

*All Grade 3 and 4 platelet count abnormalities occurred 1 day after immunization (two episodes with dose 1, 1 with dose 2, 1 with dose 3). Preceding values normal in all cases. Follow-up tests also normal in all cases, performed after 4 days (Grade 3), 5 days (Grade 3 and Grade 4), 6 days (Grade 3) and 21 days (Grade 2). There were no repeat episodes of decreased platelet counts with subsequent dosing. Toxicity scale criteria: Grade 2 creatinine elevation: > 88-141 µmol/L; Grade 2 platelet count: 51-75,000/µL Grade 3 platelet count: 25-50,000/µL; Grade 4 platelet count: < 25,000/µL

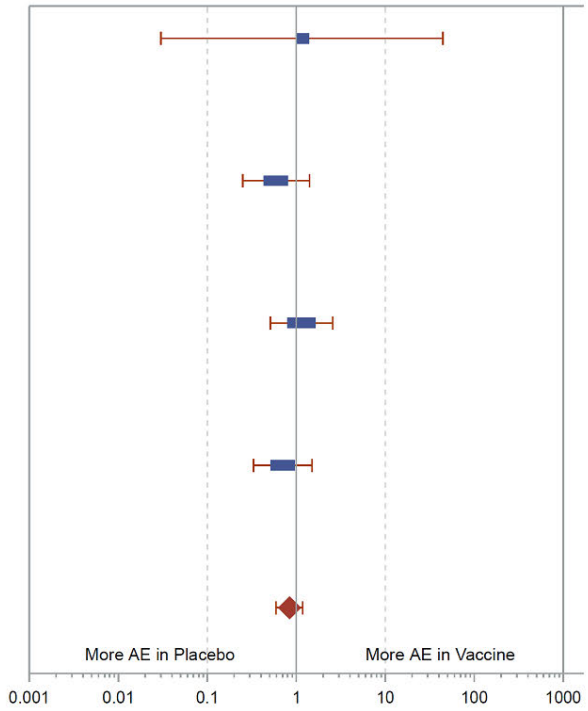
These findings in pediatric studies are illustrative of the findings in all trials of PfSPZ Vaccine, including all nine randomized, double-blind, normal saline placebo-controlled trials that have been completed and unblinded. None of these trials has shown any differences in the rates of any AEs between vaccine recipients and controls after unblinding (#5, 7, 8, 9, 10, 12, 14, 15 in **Table 3**) (Jongo S, unpublished; Sissoko M and Healy S, unpublished; Oneko M and Steinhardt L, unpublished)(3-10) except for one study where there was an excess of myalgia in the vaccine group (#10 in **Table 3**). Most importantly, there were no age or dose effects on the rates of AEs with doses as high as $1.8x10^6$ PfSPZ in infants and young children (3, 10). There also has not been an increase in AEs with repeated dosing (i.e., the rates of AEs after second or third doses have been similar to the rates after first dose). It is thus not clear that PfSPZ Vaccine has consistently caused *any* adverse reactions at the doses tested to date. In addition, there have been no allergic reactions linked to the vaccine. A few volunteers have developed urticarial rashes in the days following immunization, but in no cases have these rashes been clearly linked to the vaccine.

A meta-analysis of AE data is shown for children using forest plots of total solicited AEs in vaccinees and placebos in the randomized, double-blind, placebo-controlled trials analyzed to date (**Figure 8).** In all cases, 95% confidence intervals cross a ratio of 1, indicating no differences between vaccinees and controls.

Figure 8. Effect of vaccine on incidence of any solicited systemic adverse events per study subject in randomized, double-blind, placebo-controlled trials of PfSPZ Vaccine in infants and children aged 5 months to <18 years in sub-Saharan Africa.

Trial sites included: Bagamoyo, Tanzania (BSPZV2); Siaya, Kenya (KSPZV1b); and Bioko Island, Equatorial Guinea (EGSPZV2). #Vac. is the number of subjects enrolled in the vaccine arm in each study; # Plac. is the number of subjects enrolled in the NS control arm of each study. Numbers in parentheses are the number of subjects experiencing the AE after any of the injections administered. V = vaccine recipients, NS = normal saline recipients, OR = odds ratio, LCL = lower confidence limit, UCL = upper confidence limit. Weight is based on sample size and is represented by the width of the blue bar.

Trial	Country	Year	# Vac. (# AE)	# Plac. (# AE)	Risk Ratios	Lower 95% C.I	Upper 95% C.I.	Study Weight* (%)
BSPZV2	Tanzania	2015	51 (1)	24 (0)	1.18	0.03	44.57	14
KSZPV1	Kenya	2016	112 (10)	53 (8)	0.59	0.25	1.41	27
KSZPV1	Kenya	2016	252 (24)	84 (7)	1.14	0.51	2.56	32
EGSPZV2	EG	2016	50 (14)	15 (6)	0.70	0.33	1.50	27
Overall			**465 (49)**	**176 (21)**	**0.84**	**0.59**	**1.18**	**100**



- **Weighting = Modified Variance**
- **I^2 = 5.5% (0-36)**
- **P-value overall = 0.15**

Against this background of favorable safety data, two SAEs have occurred that were assessed as possibly related to the administration of PfSPZ Vaccine. In each case the potential association was based on timing without a clear pathophysiological link. Both of these SAEs occurred in the EGSPZV2 trial in Equatorial Guinea (#14 in **Table 3**) and led to halting of the study while the SAEs were reviewed by the Data and Safety Monitoring Board (DSMB). Direct causality could not be established, and in each case the DSMB, all associated IRBs, the regulatory agency in Equatorial Guinea and the US FDA approved resumption of the trial.

Seizure in 15-year-old boy: In the first case, a 15-year-old boy with no known history of seizures experienced a seizure 3½ hours after receiving a third dose of $1.8x10^6$ PfSPZ of PfSPZ Vaccine. The boy had a normal head CT scan and a normal head MRI. However, an electroencephalogram (EEG) revealed abnormalities consistent with generalized epilepsy. A consulting neurologist posited that the general inflammatory response to the vaccine may have lowered the seizure threshold in this individual, whose EEG was consistent with a predisposition to seizures, and whose sister had a history of a complex febrile seizures. Given that the boy experienced only one seizure, even though he received three identical doses of PfSPZ Vaccine, it is not possible to know whether the PfSPZ non-specifically affected the seizure threshold in this boy, as can occur with inebriation or sleep loss, or whether the timing of the seizure was coincidental. The boy was not treated with anti-epileptics and was interviewed one year after the seizure and reported good health without any additional seizures.

This is the only seizure occurring in temporal proximity to the injection of PfSPZ Vaccine. The same $1.8x10^6$ PfSPZ dose of PfSPZ Vaccine has been administered by DVI to 150 other children ranging in age from 5 months to 17 years, including 92 infants, most of whom received three injections with this dose, without any seizures linked by timing to the vaccine. Three infants in the KSPZV1 trial of PfSPZ Vaccine (#12= in **Table 3)** conducted in Kenya developed epilepsy during their 16-month participation in the trial, but the seizures did not occur at the time of vaccination, and the rate of new epilepsy diagnoses in the trial was not different from the background rate in this population. All three infants are now seizure-free and off medication, but one remains with developmental delay. Although 23 febrile seizures and 7 non-febrile seizures occurred in the 296 infants receiving injections with PfSPZ Vaccine in the KSPZV1 trial (versus 5 febrile seizures in 105 placebo recipients), none occurred proximally to the injection of PfSPZ Vaccine as had happened with the boy in Equatorial Guinea. However, 10 of the 23 febrile seizures occurred in the $1.8x10^6$ PfSPZ dose group, while only 5 occurred in the placebo group suggesting a trend of increased frequency in the vaccine group. Most of these seizures occurred during acute malaria infections spaced out during the 12 months of surveillance following immunization, and their frequency may have been increased in vaccinees secondary to the rebound malaria seen in the vaccine groups in that study (see below). Parents were contacted a year after the study ended, and asked about seizures, and the rates were no different among the three vaccine and one placebo groups.

Given these considerations, as a precaution until more data are available, Sanaria recommends excluding any individual with a history of seizures other than simple febrile seizures as a young child from participation in clinical trials of PfSPZ-based products. It is expected that with further clinical experience this restriction may be lifted.

Fetal loss in 19-year-old woman: In the second possibly related SAE, a 19-year old woman became pregnant at about the same time as she received a first dose of $2.7x10^6$ PfSPZ of PfSPZ

Vaccine. An ultrasound at 9 weeks gestational age by last menstrual period showed a 6-week sized embryo without cardiac activity. Because about a quarter to a third of all pregnancies end in spontaneous abortion, it is unclear whether the administration of PfSPZ was related to the loss of this embryo. As was the case in the EGSPZV2 trial, avoidance of pregnancy remains an important inclusion criterion for trials of PfSPZ Vaccine.

Rebound malaria: A finding from the KSPZV1 trial in 5-11-month-old Kenyan infants was that as the efficacy of the vaccine waned, the frequency of malaria infections increased in vaccinees relative to the controls. This evolution, called "rebound," has been seen with the RTS,S malaria vaccine and with malaria chemoprophylaxis (21-23). The hypothesis proposed to explain rebound is that vaccine- or drug-induced protection delays the onset of naturally acquired immunity (NAI), which depends upon repeated exposure to malaria. While the placebo group is developing NAI, experiencing a progressively diminishing risk of clinical disease, the vaccinees lag behind based on their lower infection rates and reduced exposure to parasitemia. As a result, they may subsequently experience rates of parasitemia and Pf *malaria with symptoms* that exceed those in controls once vaccine-induced protection has waned. A pattern consistent with this hypothesis was seen in the KSPZV1 trial in Kenyan infants, where vaccinees had less exposure to parasitemia than placebo recipients during the first 3 to 6 months after vaccination (Oneko M and Steinhardt L, unpublished). Despite the excess rates of Pf *malaria with symptoms* in vaccinees toward the end of the 12-month follow-up, the total number of clinical episodes was about the same in placebo and vaccine groups over the full year that malaria parasitemia was monitored. In Mali, an area of highly seasonal malaria transmission, it is very unlikely that rebound malaria cases will occur during the dry season after the scheduled surveillance period. In addition, if significant efficacy is seen on this trial it is likely that the study will be extended for an additional malaria transmission season to assess the protective efficacy of a booster dose, and monitoring for malaria cases would also occur for participants who re-enroll.

Epstein-Barr virus reactivation: US FDA requested that serological studies of EBV capsid antigen be performed in previous pediatric trials of PfSPZ Vaccine. The concern was based on the association between Pf *malaria*, latent EBV reactivation, and endemic Burkitt lymphoma (24). Since PfSPZ Vaccine contains malaria parasites, exposure following injection might reactivate EBV, even though this reactivation is thought to be precipitated by blood stage parasites, not by sporozoites. If this were to occur, it should be reflected in increasing antibodies to EBV capsid antigen following immunization. The data from the BSPZV2 trial did not show any effect of immunization on antibody levels in any of the 5 age groups studied in that trial (adults, teenagers, older children, younger children and infants). Serology assays are currently being performed to test the same hypothesis using samples from EGSPZV2 and KSPZV1.

Summary: The side effect profile of PfSPZ Vaccine recorded in 19 Phase 1 and 2 clinical trials has been benign, and not been different from that of NS placebo in trials including this control in those studies that have been unblinded. However, the side effect profile could evolve with the performance of trials which have increased power to detect small differences in the rates of AEs between vaccinees and placebo recipients, and which have a higher likelihood of revealing uncommon adverse effects due to larger sample sizes. Until such studies can definitively rule out side effects, the study Sponsor, Sanaria continues to describe the signs and symptoms that are typically caused by less well tolerated vaccines, or by malaria, as theoretical concerns. These include:

Local reactions: injection site pain, tenderness, erythema, swelling, induration, pruritus, bruising, bleeding, lymphangitis, regional lymphadenopathy, and arm motion limitation.

Systemic reactions: measured fever, subjective fever, headache, malaise, fatigue, chills, rigors, sweats, myalgia, arthralgia, dizziness, nausea, vomiting, abdominal discomfort, anorexia, asthenia, insomnia, anxiety, confusion, cough, shortness of breath, chest pain, palpitations, and allergic reactions such as rash, urticaria, pruritus, angioedema, and anaphylaxis, the latter potentially resulting in death, and unexpected AEs that have not previously been reported.

Although it has never happened, there is also the theoretical risk of breakthrough infection due to inadequate attenuation.

Normal Saline

The amount of normal saline used in this study is small (< 1 mL) and has been well tolerated, including in previous PfSPZ Vaccine studies. Overall, most AEs reported with the use of normal saline have been reactions that may have occurred because of administration technique and have been local injection reactions as noted above in **Section 2.3.1.2**.

Antimalarial Medications (AL)

Subjects will be treated with a registered, oral, proven, and highly efficacious treatment during the course of this study per standard clinical practice. These medications will be tracked closely, similar to study specific products.

Artemether/lumefantrine (AL) is the first-line anti-malaria drug regimen currently recommended for use by the Malian Ministry of Health to treat cases of acute malaria disease in adults and children.

AL 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., >10%) in children less than 16 years of age are headache, anorexia, fever, and cough. 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.

The frequency of dosing on this trial (two treatment courses spaced approximately one month apart) is not expected to increase the risk of side effects.

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

SARS-CoV-2 Transmission

This trial will likely be conducted under the conditions of ongoing SARS-CoV-2 transmission in the local community. As of 10 April 2021 there have been 11,393 confirmed cases and 404 deaths due to COVID-19 in all of Mali. However, these numbers could underestimate the true COVID-19 infection rate and it is assumed that by bringing community members together at a clinical trial site to receive immunizations and check-ups, the conduct of a clinical trial could in theory augment local transmission. To prevent this, and to protect clinical staff members and

study participants, the clinical team will strictly follow mask wearing, hand-washing and social isolation measures in accordance with local public health guidelines and site Standard Operating Procedures.

Risks to Privacy

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subject's PHI. All study records will be kept in a locked file cabinet or maintained in a locked room at the site. Electronic files will be password-protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected. Any publications from this trial will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the site for quality assurance (QA) and data analysis include groups such as the local or central IRB, NIAID, and the Food and Drug Administration (FDA).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This web site will not include information that can identify subjects.

Unknown Risks

There may be other risks, discomforts, or side effects that are unknown at this time.

3.3.2 **Known Potential Benefits**

PfSPZ Vaccine has been shown in previous studies to provide some protection to the individual from malaria infection over a 24-week period during the malaria transmission season. For children who receive the study vaccine, there is a potential of direct benefit either from protection from patent parasitemia and/or Pf *malaria with symptoms* or from decreased poor outcomes related to secondary anemia.

Subjects who receive placebo will not have personal, direct benefits from participation during the course of the trial ascribable to receiving an investigation vaccine. However, the risk to subjects receiving placebo is minimal, and all participants will have close follow up during their study participation and have study evaluations, which may provide some benefit. Even without any personal direct benefits, subjects may contribute to a societal benefit through the improvement of our understanding of these vaccines and how they can be utilized to improve maternal and neonatal malaria outcomes.

Administration of artemether/lumefantrine on this trial to clear parasitemia has the potential to directly benefit participants, similarly to seasonal malaria chemoprevention.

3.3.3 **Assessment of Potential Risks and Benefits**

The risks and benefits described above will be reviewed by the relevant Investigational Review Boards for an ethical determination. It is anticipated that this protocol will be classified as greater-than-minimal risk for vaccine recipients and minimal risk (or a minor increase over minimal risk) for placebo recipients. The protocol's acceptability is enhanced by strategies for risk mitigation, which are described here.

To increase safety during the trial, subjects will be monitored closely, including the measurement of vital signs, the performance of focused physical examinations and hematology assessments,

and the collection of AEs. SAEs will be monitored throughout the trial. Any medical conditions that develop will be evaluated and treated to the extent possible. Study doctors will be available 24 hours a day, 7 days a week to provide care.

Because the study will be performed in a malaria-endemic area, malaria infection is a risk during the trial, and subjects will be told to contact research personnel if malaria symptoms develop. During every encounter with clinical staff, subjects will be asked actively about the symptoms of malaria. If symptoms are present, a blood sample for TBS will be obtained (if not already scheduled) and will be examined for the presence of parasites. Those found positive will be promptly treated. Study subjects will be followed during the three-day administration of antimalarial treatment to assure clinical resolution.

To minimize the risks associated with skin puncture, immunizations will be preferentially given in a cleaned and disinfected area of the upper extremity via a sterile 25-gauge needle and syringe by a skilled medical provider using aseptic technique, thus minimizing any chance of extravasation or infection. Blood drawing will likewise be performed with a sterile needle and syringe after cleaning and disinfecting the phlebotomy site.

Emergency equipment and supplies to allow urgent endotracheal intubation, administration of oxygen, epinephrine, antihistamines, corticosteroids and other drugs, will be available at any site where immunization is performed to treat any acute allergic reactions that might occur. Staff will be available who are trained in pediatric advanced cardiac life support or equivalent. A defibrillator and cardiac monitor will be available for use.

In order to decrease the risk of a breach of subject confidentiality, all study procedures will be conducted per GCP guidelines. Personal identifiers will not be used in publications or communications from this research study. Rather, a coded and unique study number will identify specific details related to each study subject in an effort to maintain their confidentiality. All study-related information will be stored securely in locked areas with access limited to study staff. All databases will be secured with a password-protected access system, and subjects' study information will not be released without their written permission, except as necessary for monitoring compliance with legal or regulatory requirements or obtaining medical consultations to provide medical care.

According to Malian Law No 09-059, when a research participant is a minor with either direct individual benefit or without direct individual benefit, their legal representative(s) and/or guardian(s) must provide consent. In addition, the research must not present a serious foreseeable risk to participants who are minors and, if the study is to be conducted without direct benefit to participant(s) who are minors, the research must be useful to people with the same age, illness or disability characteristics and provide results that cannot be achieved otherwise. We believe this study satisfies those criteria.

Summary:

We propose that for vaccine recipients, participation in this trial involves greater than minimal risk but a well-justified prospect of direct benefit from the vaccine while for placebo recipients participation in this trial constitutes no greater than minimal risk based on the benign nature of injection of normal saline through a small-gauge needle.

The benign side effect profile of PfSPZ Vaccine and the risk mitigation procedures described above support a favorable risk-benefit assessment for the conduct of this trial, especially given the potential societal benefit should this research study lead to a safe and effective malaria vaccine.

4. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To describe the safety of PfSPZ Vaccine in children with respect to the occurrence of possibly, probably, or definitely related serious adverse events (SAEs).	Proportion of vaccinees compared to controls experiencing related SAEs from V1 to 26 weeks after V3	SAEs potentially represent the most important adverse vaccine reactions.
To measure VE against first episode of Pf *malaria with symptoms* by time-to-event analysis.	VE computed as one minus the estimated hazard ratio (HR) for first episode of Pf *malaria with symptoms* from 2 weeks after V3 to 26 weeks after V3 in the modified ITT (mITT) population.	Time-to-event analysis is standard in the malaria vaccine field and takes into account how many subjects develop first Pf *malaria with symptoms* and time for this to happen.
Secondary		
To assess the safety of PfSPZ Vaccine with respect to the occurrence of unsolicited AEs, laboratory abnormalities, solicited AEs.	Safety and tolerability in children: - The differences in proportions of vaccinees compared to controls experiencing unsolicited AEs from the time of V1 to 14 days after V3 (or last immunization). - The differences in proportions of vaccinees compared to controls experiencing laboratory	These endpoints could potentially reveal the occurrence of adverse vaccine reactions.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	abnormalities from time of V1 to 14 days after V3. - The differences in proportions of vaccinees compared to controls experiencing solicited AEs during the 7 days following each immunization.	
To measure VE against first episode of Pf *malaria* (parasitemia) with or without associated symptoms by time-to-event analysis.	VE computed as one minus the estimated hazard ratio (HR) for first Pf *malaria* (parasitemia) detected by TBS, from 2 weeks after V3 to 26 weeks after V3 in the mITT population.	TBS is the standard diagnostic method for detecting parasitemia. Time-to-event analysis is standard in the malaria vaccine field for measuring VE taking into account how many subjects develop first Pf *malaria* and time for this to happen.
To measure antibody responses to Pf circumsporozoite protein (CSP) and their association with protection.	Antibody levels to PfCSP by standardized ELISA comparing protected and unprotected vaccinees and controls.	This has been the best correlate of PfSPZ Vaccine take as measured by the induction of protective immunity.
Exploratory		
To assess the immune response to PfSPZ Vaccine in children.	Humoral and cellular immune responses to PfSPZ Vaccine	These assays may assist with understanding the mechanism of protection or identification of immune correlates.
To assess genetic relatedness of the PfSPZ Vaccine parasite strain to malaria infection parasites.	Genotyping of peripheral blood *Pf* parasites	These assays may assist with understanding the mechanism of protection.
To measure VE using other outcome measures such as	Additional measures of vaccine efficacy:	Different measures of malaria-related morbidity are

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
against all episodes of Pf *malaria with symptoms* and/or Pf *malaria* rather than against the first episode only, or using proportional analysis, or using ITT or ATP populations, or severity of malaria infections or hemoglobin concentration.	- VE against all episodes (rather than first episode) of Pf *malaria with symptoms* and Pf *malaria* by Cox regression analysis - VE computed by proportional (binary) analysis (1 – risk ratio) for first episode of Pf *malaria* detected by TBS, from 2 weeks after V3 to 26 weeks after V3 in the ITT, mITT and ATP populations (whole group and subgroups) - VE computed as one minus the estimated HR for first episode of Pf *malaria* detected by TBS, from 2 weeks after V3 to 26 weeks after V3 in the ITT and ATP populations (whole group and subgroups) - VE for Pf *malaria* measured by and nucleic acid detection in children from 2 weeks after V3 to 26 weeks after V3 (time to event, proportional/binary) - VE by both time-to-event and proportional/binary analyses for preventing and delaying and by relative risk analysis for preventing first episodes of Pf *malaria with symptoms* with - parasite density > 500 parasites/μL, or - parasite density > 5000 parasites/μL	important to understand the spectrum of VE.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	- or of Pf *malaria with symptonms* characterized by: - grade 3 signs or symptoms, - fever and convulsions, or - hospitalization, or - other clinical measures in vaccinees vs. controls. - Rates of anemia (grade 1, grade 2, grade 3) in vaccinees vs. controls.	
To explore the relationship between detectable parasitemia prior to vaccination and VE.	Retrospective malaria qPCR values taken prior to vaccination	Analyzing quantitative malaria PCR results across several pre-vaccination timepoints may allow for assessment of the impact on VE of low-level parasitemia that would otherwise be below the threshold of detection at a single timepoint.

5. STUDY DESIGN

5.1 OVERALL DESIGN

This phase 2 study will enroll healthy Malian children between 6 and 10 years of age residing in Bancoumana and surrounding villages to participate in a randomized, double blind, placebo-controlled study to assess the safety, immunogenicity and protective efficacy of PfSPZ Vaccine. Participants will be immunized with a 3-dose series of 9.0 x10^5 PfSPZ of PfSPZ Vaccine or normal saline (placebo) at 1, 8, and 29 days. Subjects will be screened for eligibility for enrollment. Enrollment will begin with AL dosing approximately 4 weeks prior to their first dose of vaccine. Volunteers will be randomized into two arms (1 vaccine arm, 1 control arm) in a 1:1 ratio.

Vaccinated subjects and controls will then be followed for safety and assessment for malaria infection during the subsequent malaria transmission season.

~268 children between the ages of 6 and 10 years old inclusive will be enrolled as follows:

> **Arm 1 (PfSPZ Vaccine):** (n = ~134) children ages 6 – 10 will receive three doses of PfSPZ Vaccine ($9.0x10^5$ PfSPZ) via direct venous inoculation (DVI) at 1, 8, and 29 days
>
> **Arm 2 (normal saline):** (n = ~134) children ages 6 – 10 will receive normal saline via DVI at 1, 8, and 29 days

All subjects will receive artemether-lumefantrine (AL) approximately 1-4 weeks before the first and third dose of PfSPZ Vaccine or normal saline for clearance of *Pf* parasitema.

NB.: the second round of AL clearance prior to third vaccinations may or may not proceed depending on the timing of the beginning of the rainy season and the timing of malaria transmission in the community and will be decided by the Mali PI.

Vaccinated participants and non-vaccinated controls will be monitored for development of Pf *malaria with symptoms* and Pf *malaria* (parasitemia) through the natural malaria transmission season to estimate vaccine efficacy (VE). During the surveillance period, both active and passive surveillance will be used to identify Pf *malaria with symptoms* (see section 9.7). Blood smears will be made at any time a participant presents with a clinical syndrome consistent with malaria and read in real time, with all infections treated. Twenty-eight days later, these subjects will resume surveillance to make it possible to count all episodes of malaria with symptoms (exploratory objective). Parent/guardians will be encouraged to report to the clinic any time that a child is ill.

In addition, blood smears will be made every four weeks in all participants as active surveillance for Pf *malaria* (parasitemia). However, to avoid confounding the primary clinical endpoint, these blood smears will be reported retrospectively at the end of the primary surveillance period.

This study will not interfere with routine standard-of-care for children. Participants will receive medical care through the local health clinic, including standard immunizations and seasonal malaria chemoprophylaxis if indicated, per the standard of care in Mali. The study will support the clinics by providing acute care assessments, ensuring supply of RDT and blood smears for malaria diagnosis, drugs for malaria treatment and prevention, and neurodevelopmental screening. During the study visits, the team will encourage families to continue consistent follow-up with their pediatric providers and will coordinate any acute care in consultation with the local pediatrician as needed.

Indoor residual spraying (IRS) is performed in only about 3 of the 75 districts in Mali, and this does not include Bancoumana. Therefore, it is not expected that IRS will affect outcomes in this study. In 2018, bednet use was 82.2% in the Koulikoro region of Mali where Bancoumana is located. It is expected that randomization will balance out bednet use between the study groups. However, participants will be evaluated at routine visits with regard to bednet use, so that a subgroup analysis can be performed taking bednet use into consideration.

5.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

5.2.1 Justification for Dose

Multiple trials in US and African adults and in African children (as summarized in **Table 3**) have been completed to date with reassuring safety results at different PfSPZ Vaccine doses (up to $2.7x10^6$ PfSPZ) and dosing regimens. These trials include 7 trials in Europe and the United States as well as 13 in malaria exposed adults, children and infants in Africa. Clinical trials have assessed as few as two doses and as many as six doses. Numerous regimens have been carefully assessed, including widely spaced (weeks 1, 9, 17), condensed (days 1, 8, 29), and combination regimens (multi-dose prime with late boost). Ten different dose strengths have been tested, starting at $7.5x10^3$ and increasing to $2.7x10^6$ PfSPZ per injection.

In 4 of the field trials conducted in Mali and Burkina Faso, PfSPZ Vaccine administered after antimalarial pretreatment using various dosages and schedules (including a 28-day regimen) has demonstrated efficacy against new *Pf* infection of up to 56% in time-to-event analyses over periods ranging from 20-24 weeks during the malaria season. These findings are discussed in more detail in **Section 2.2.1.1** above. In a trial from Burkina Faso, PfSPZ Vaccine showed significant efficacy when subjects were followed over two years.

A major finding has been that an accelerated 28-day regimen (Days 1, 8 and 29) provides protective efficacy which is as good as or better than any of the more widely-spaced regimens. Key findings from the 20 trials conducted with PfSPZ Vaccine include: 1) two doses are not sufficient (maximum VE 67% against homologous CHMI), 2) three doses are as good as 5 doses, 3) multi-dose priming is important, 5) priming with Days 1 and 8 appears to be as good as or better than priming with Days 1, 3, 5 and 7, and 6) dose escalation after a certain threshold appears to have no additional benefit. The dose of $9.0x10^5$ PfSPZ administered on Days 1, 8 and 29 has been down-selected by Sanaria as the dose to be brought into Phase 3 testing and is the dose and regimen that will be used in this trial.

5.2.2 Rationale for Age Groups Selected

Naturally acquired immunity (NAI) to malaria develops in those living in endemic areas as a result of repeated exposures to malaria parasitemia (28). It includes an anti-disease component (ability to sustain a given density of parasitemia without signs or symptoms) and an antiparasite component (ability to suppress parasitemias to lower densities)(29). The rate with which individuals develop NAI varies with the level of exposure across a range extending from high exposure rates (multiple infections per individual per year) where malaria-related morbidity and mortality are concentrated in the youngest age groups (e.g., < 5 years of age) because all older individuals have developed some level of NAI, and low exposure rates (multiple years between infections) where malaria-related morbidity and mortality are spread across all age groups including adults because most individuals fail to develop functional NAI.

A cohort study of the community dynamics of malaria transmission (including the incidence of clincal malaria) was recently conducted in Bancoumana, Mali, and enrolled a total of 830 volunteers aged 6 months to 65 years of age (Sissoko et al, unpublished data). Study participants were seen monthly and at the time of any illness by study clinicians. Malaria blood smears and/or rapid diagnostic tests (RDTs) were performed upon observation of clinical symptoms to confirm clinical malaria before treatment initiation. Incidence data on malaria with symptoms collected from February 2018 to January 2020 are presented in **Table 10**.

Table 10. Incidence of symptomatic malaria infection per person per year in Bancoumana, Mali by age and season.

Age (years)	2018			2019		
	Dry	Wet	Overall	Dry	Wet	Overall
0.5 – 4	0.02	0.34	0.37	0.10	0.50	0.60
4 – 10	0.07	0.77	0.84	0.16	0.86	0.99
11 – 17	0.06	0.86	0.92	0.18	0.90	1.08
18 – 65	0.03	0.47	0.50	0.07	0.44	0.52
All ages			0.68			0.83

The highest rates of malaria with symptoms occurred during the wet season (from July to December) in the 4 -10- and 11–17-year-old age groups, approaching 1 episode on average per year per person in these groups, with the preponderance of infections occurring during the wet season. The disproportionately low incidence in under-fives could be explained by seasonal malaria chemoprevention distributed during the high malaria transmission season (July/August to October/November). The disproportionately low incidence in adults likely results from well-developed NAI. Overall, these data suggest that school age children have less well developed NAI and would particularly benefit from an effective malaria vaccine.

The evolution from clinically susceptible to clinically protected is gradual and in Bancoumana this transition seems to begin in the early teenage years. For this protocol, the dividing line between age 10 and 11 has been chosen as it roughly approximates the development of partial clinical immunity, although many older teens and adults remain susceptible to clinical illness due to malaria. At the lower end, a cut-off of 6 years is used to exclude children expected to receive SMC as this may impact the primary endpoint of Pf *malaria with symptoms*. While SMC is recommended for children 5 years of age and under, in practice it is not unusual for children up to a year over the age of 5 to receive SMC doses in the areas where this trial will be conducted. Thus, children 6 years of age who do take SMC will be excluded from the study.

5.2.3 Rationale for Antimalarial Treatment prior to V1

Malaria parasitemia can cause immunosuppression, even at densities too low to be detected by TBS or qPCR. The most revealing data come from a recent clinical trial of PfSPZ-Cvac (a different Sanaria vaccine approach), where injection of PfSPZ into parasitemic volunteers inhibited the development of sterile protective immunity. Zero of nine volunteers undergoing CHMI were protected following immunization when immunization was superimposed on parasitemia at densities too low to be identified by TBS, whereas 6/8 volunteers were protected

in the same trial when the injection day was shifted forward by two days to avoid immunizing during parasitemia (Jackson L, unpublished data). Furthermore, immunosuppression has also been demonstrated in Kenyan children (30), and has been mechanistically explored in murine studies.

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

Table 12. VE against Pf infection in MLSPZV3 and MLSPZV4

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

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

When all eight trials are considered together, the difference in protection between trials with and without pre-treatment is statistically significant (Fisher's exact test p=0.018). For this reason, all field trials of PfSPZ vaccines, if taking place in endemic areas where study subjects may be chronically infected with malaria, include treatment with a curative antimalarial drug regimen prior to the first and perhaps third vaccinations. As further confirmation, Sanaria will be conducting a trial in 2-10 year-old children in Equatorial Guinea in 2022 in which participants will be randomized to receive or not receive pre-treatment using a randomized, double-blind design (Richie, personal communication).

Therefore, to reduce the risk of immunosuppression, all study subjects will be cleared with a full six-dose, three-day treatment regimen of AL prior to V1. 14 days is about 2 to 4 half-lives of the longer acting drug, lumefantrine, which has a half-life of 3-6 days. This gap should be sufficient because lumefantrine is not known to have activity against sporozoites or liver stage parasites. The 14-day gap will also provide some time for the immune system to recover from the effects of pre-existing parasitemia, although the actual time required is not known.

When PfSPZ Vaccine immunization is done when there is ongoing malaria transmission risk, the three-day treatment course with AL is administered a second time prior to the third vaccine to avoid the immunosuppressive effects of parasitemia just described, in case any participants may have acquired a naturally transmitted malaria infection during the immunization period. However, because immunizations will be done during the dry season, when the risk of malaria transmission is essentially nil in this region of Mali, a second AL administration will not be done.

5.2.4 **Rationale for Parasitemia Threshold Selected**

To comply with a recommendation by the FDA, we have used a data collected in a survey of Community Dynamics of Malaria Transmission and Mosquito Feeding in Bancoumana, Mali (NCT03304704) conducted from February 2018 to September 2020 which should be applicable to the proposed study population. This Community Survey encompassed three dry seasons (2018, 2019 and 2020) and the full rainy seasons of 2018 and 2019. The available data set ended early in the rainy season of 2020. In the Community Survey study, participants ranging in age from 2 months to 87 years were seen in scheduled visits at the clinic once a month, at which time they were questioned about symptoms, examined for axillary temperature, finger-pricked for a malaria thick blood smear, classified according to diagnosis if clinically ill, and treated as needed. Participants were also encouraged to report to the clinic outside of scheduled visits in case of acute illness, in which case the same assessments were made. Any density of parasitemia identified, if associated with clinical signs or symptoms consistent with malaria, was classified as "malaria." Note that fever was not a requirement for a diagnosis of clinical malaria. This followed the Malian Ministry of Health directive for identifying a case of clinical malaria:

Mali Ministry of Health Case Definition for uncomplicated clinical malaria requiring treatment:

1. Parasitemia confirmed by a biological examination, either microscopy or rapid diagnostic test *plus*
2. Fever (axillary temperature ≥ 37.5 ° C) / history of fever *and/or*[3] headache, myalgia, joint weakness, digestive disturbances, chills, body aches.

If a participant was positive for parasitemia as confirmed by thick blood smear and had clinical signs or symptoms but these were not consistent with malaria, the individual was not classified as having clinical malaria but was given an alternate diagnosis. If a participant was positive for parasitemia, did have clinical signs and symptoms consistent with malaria, but additionally had signs or symptoms referrable to a second diagnosis, the individual was assigned both diagnoses, e.g., malaria + rhinitis. If parasitemia was present in the absence of signs or symptoms, the diagnosis "asymptomatic parasitemia" was assigned. All of these assignments were collected as

[3] French language version uses "avec" (with) but this is interpreted in practice as "and/or" meaning that fever is not required for a diagnosis.

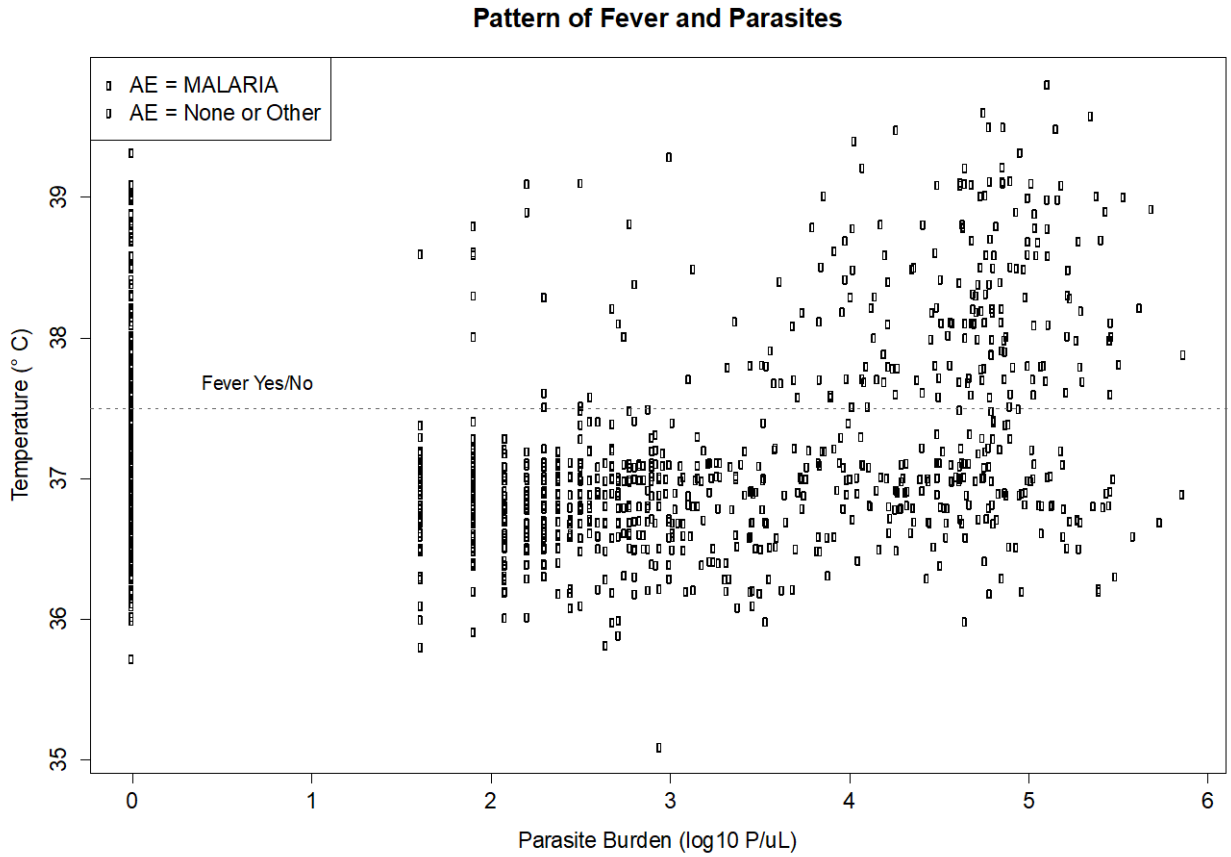
"adverse events (Aes)" and visits to the clinic where an AE was identified were called "AE visits."

To address the Agency's recommendation for selecting a parasitemia threshold, we examined the subset of data from the Community Survey study restricted to 6- to 10-year-olds living in Bancoumana, our proposed study site. This included 277 children who were seen on average 20.3 times (range: 1 to 43) totaling 5626 clinical visits. Of those, 221 children had at least one AE visit, where they were either positive for parasites or classified as having some diagnosis, including clinical malaria ("malaria"). On average, each AE child had 7.3 AE visits (range: 1 to 23). There were 21 cases where one AE visit was assigned two diagnoses. In 15 of those cases, one of the diagnoses was clinical malaria. The most common presentation of two diagnoses was malaria plus rhinitis. Thus, this dataset is appropriate to assess parasitemia thresholds and their potential impact on mis-identifying acute malaria based on symptoms that may arise from other non-malarial illnesses.

For the 277 children in the 6- to 10-year-old age group, 5990 blood smears were collected, of which 964 smears showed detectable parasitemia in 195 unique individuals. Of the 964 positive smears, 582 (60.4%) occurred without reported symptoms, 382 (39.6%) were associated with at least one symptom, 370 were reported as malaria, 10 were reported as another diagnosis, and 15 were reported as malaria + another diagnosis. Additionally, 5026 child visits were negative for parasitemia by RDT and/or smear. Of those, 4419 (87.9%) occurred without any reported symptoms, and 607 (12.1%) were associated with at least one symptom.

The association between parasitemia density (x-axis) and axillary temperature (y-axis) in the 370 AE visits (165 children) classified with a diagnosis of malaria is shown in Figure 9. Although temperature was weakly but significantly associated with parasite density in these children (Pearson R=0.18, p=0.001), many children with malaria did not have fever at both high and low parasite densities. Fever was relatively less common in these non-malaria cases. A significant number of children with no parasitemia also had fever.

Figure 9: Pattern of Fever and Parasites

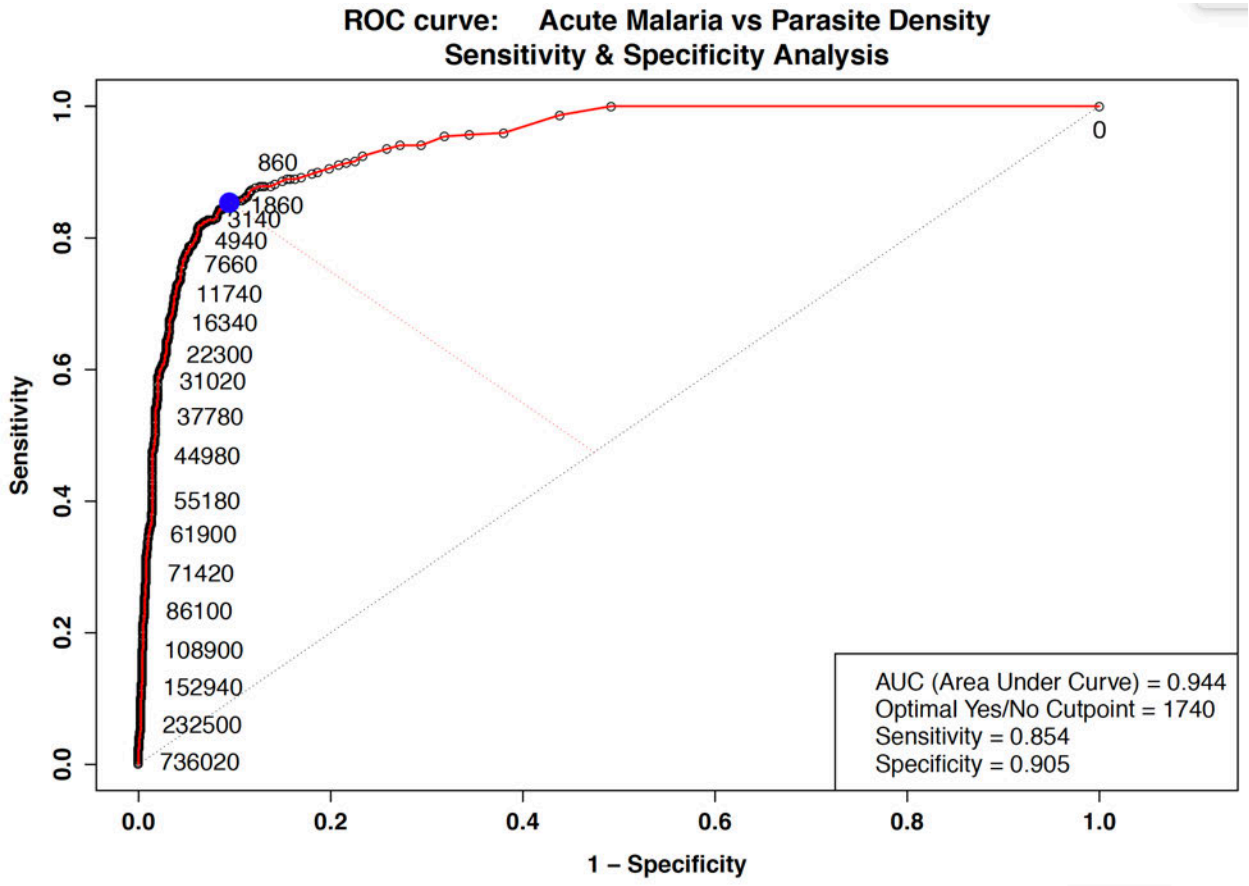


As described below, we used these data to identify a parasitemia threshold selected to increase the specificity with which clinical malaria could be diagnosed in this population without significant loss of sensitivity. Two approaches were used. The first was to generate a Receiver Operator Characteristics (ROC) Curve using data from all clinic visits with a diagnosis including the diagnosis of asymptomatic parasitemia. The second was to calculate the fraction of fevers that could be attributed to the presence of parasitemia, using data from all clinic visits. We selected fever because in our proposed clinical trial, the presence of fever will be a component of the case definition for clinical malaria.

ROC Curve: Clinical malaria diagnoses and parasitemia measurements from 1604 AE visits over the entire study period were inputs to the R ROC function (package ROC [1]) to generate an ROC curve (Figure 10). By definition of ROC, the parasitemia threshold corresponding to the point on the curve that is farthest from the X=Y diagonal represents the optimal yes/no cut point discriminant value. The optimal yes/no cut point was calculated to be 1740 parasites/uL, suggesting that if no criteria other than parasitemia is available for making a clinical malaria diagnosis, then only children presenting with at least 1740 P/uL should be called clinical malaria, and those with lower levels should be considered to be asymptomatic. The area under this curve was calculated to be 0.944 indicating that the 1740 P/uL threshold will correctly identify 94.4% of visits. The ROC analysis was repeated separately for each of the 2018 and 2019 malaria

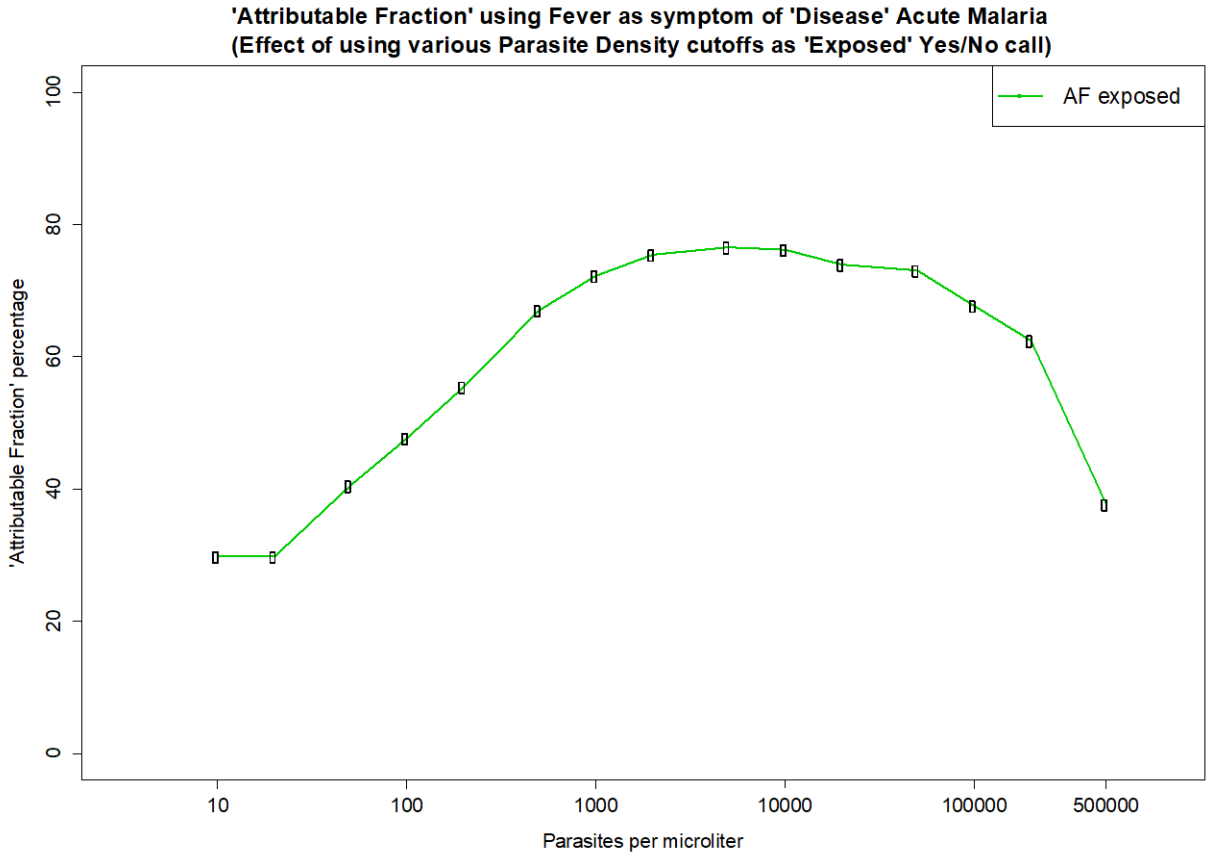
seasons (July-December), using 421 and 492 AE visits respectively, and the cut points were 2500 and 1060 P/uL, respectively.

Figure 10: Acute Malaria vs Parasite Density



Fever Attributable Fraction (AF): Fever occurred in child visits with and without parasitemia, and parasitemia occurred in child visits with and without fever, but fever was most commonly observed in visits diagnosed as clinical malaria, allowing the calculation of what fraction of fevers could be attributed to malaria. To investigate the relationship between parasitemia and clinical malaria, a series of Attributable Fraction (AF) calculations were performed, using a range of parasitemia thresholds (densities of 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10,000, 20,000, 50,000, 100,000, 200,000 and 500,000 parasites/uL), to assess how changing the parasitemia threshold changes the fraction of fevers attributable to clinical malaria. The AF was calculated as from [2]: $AF_e = (CI_e - CI_u) / CI_e$ where CI_e is the cumulative incidence of the exposed group and CI_u is the cumulative Incidence of the unexposed group. For both groups, the cumulative incidence is calculated as the proportion of child visits with fever given the number of child visits in that group. Each child visit is grouped as being either exposed or unexposed, using the visit parasitemia relative to the threshold value – visits with parasite density at or above the threshold were called exposed, while visits with no parasites or density below the threshold were called unexposed.

Figure 11: Attributable Fraction using Fever as a Symptom of Diseases



AF peaked at 5000 parasites/uL but was relatively flat from about 1000 to 100,000 parasites/uL. Based on this plateau shape, a threshold of 1000 parasites/uL appeared to be a reasonable cut-off, as higher thresholds did not significantly improve AF yet would significantly diminish the number of malaria cases and weaken the power of a clinical study.

Selection of Parasite Density Threshold for the Proposed Study: The RTS,S Phase 3 clinical trial used a threshold of 5000 parasites/uL, which appeared optimal to increase the specificity of the case definition for malaria in the infants and young children enrolled in that study. Because the densities of parasitemia in malaria endemic areas decrease progressively with duration of exposure and increasing age, due to the acquisition of naturally acquired immunity, the densities recorded in older children are lower, and in adults lower still. For example, in a cohort of Ghanaian adults who were followed longitudinally for new *Plasmodium falciparum* infection following drug clearance of parasitemia, the geometric mean parasite density in P/uL on first symptomatic parasitemia was only 451 for men (range 171 to 1190) and 347 for women (range 131 to 921). The geometric mean parasite density for asymptomatic parasitemia was 2- to 3-fold lower – 129 (range 96–169) for men and 162 (range 118–223) for women. These data would suggest a cut-off in the range of 200-300 would be appropriate for Ghanaian adults.

Our study is enrolling 6- to 10-year-olds in Mali. It is thus not surprising that our ROC curves suggest an optimal cut-off between 1000 and 2500, midway between 5000 (appropriate for infants) and 200-300 (appropriate for adults). Our AF analysis yielded similar results and

provided a rationale for moving the threshold "to the left" on Figure in order to reduce the false negative rate without significantly increasing the false positive rate. Thus, we propose to use a density of 1000 parasites/uL as a cut off in our study for the primary case definition of clinical malaria.

Relative Independence of Vaccine Efficacy from Parasite Density Thresholds: Although parasite density thresholds have been used in the past as described for RTS,S – to optimize sensitivity and specificity of the primary case definition – key field trials of malaria vaccines have shown that VE may not be significantly affected by the threshold selected. The data supporting this contention come from the RTS,S Phase 3 trial conducted at 11 sites across Africa [3] and the recently published Phase 2b trial of R21 conducted in Burkina Faso [4]. The outcome variable for both trials was clinical malaria, defined as presence of axillary temperature of 37·5°C or higher and Pf parasite density of more than 5000 asexual forms per µL. A secondary analysis was also performed in each trial using any density of parasitemia identified on thick blood smear (>0 P/uL) rather than >5000 P/ul as the threshold for diagnosing clinical malaria. The data show that whichever density was selected, VE was essentially identical. This finding is perhaps not surprising when one considers that VE is calculated by comparing the rate of clinical malaria in vaccine and placebo groups, a relative difference that might not be affected by changes in the specificity of the case definition as long as the likelihood of false positives and false negatives is the same in both vaccine and placebo groups.

In summary, although this trial is following FDA's recommendation and has a primary case definition that requires a parasite density >1000 P/uL in the 6-10-year-old target population, we will also assess a secondary case definition for parasite density >0 P/uL, and based on the results just presented, we are expecting that the outcome will be similar.

6. STUDY POPULATION

6.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual (or their legal guardians as applicable) must meet all of the following criteria:

1. Parent(s) or guardian(s) willing and able to provide consent prior to initiation of any study procedures
2. Stated willingness of parent(s) or guardian(s) to comply with all study procedures and availability for the duration of the study
3. Malaria comprehension exam completed by parent(s) or guardian(s) and passed with a score of ≥80% or per investigator's discretion
4. Healthy children 6-10 years of age at enrollment (inclusive)
5. Parent(s) or guardian(s) are 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

6.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Medical, behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the participant's parent and/or legal guardian to understand and comply with the study protocol
2. Menstruating females (in order to avoid cultural implications of further assessing pregnancy potential i.e. sexual activity in this age group)
3. Hemoglobin (Hgb), WBC, absolute neutrophils, and platelets outside the local laboratory-defined limits of normal and ≥ Grade 2 (subjects may be included at the investigator's discretion for 'not clinically significant' abnormal values)
4. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal and ≥ Grade 2 (subjects may be included at the investigator's discretion for 'not clinically significant' abnormal values)
5. Infected with human immunodeficiency virus (HIV), hepatitis B, or hepatitis C
6. Sickle cell disease by history
7. Taking or planning to take seasonal malaria chemoprophylaxis
8. Clinically significant abnormal electrocardiogram (ECG) such as abnormal QTc
9. History of receipt of the following:
    - Investigational malaria vaccine in the last 2 years
    - Immunoglobulins and/or blood products within 6 months of enrollment
    - Investigational product within 3 months of enrollment
    - Chronic (≥14 days) oral or IV corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone ≥20 mg/day or equivalent) or immunosuppressive drugs within 30 days of enrollment
    - Live vaccine within 30 days of enrollment
    - Killed vaccine within 14 days of enrollment or planned receipt of a killed vaccine within 14 days of scheduled vaccination
10. Known medical problems:
    - Pre-existing autoimmune or antibody-mediated diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia)
    - Severe asthma (defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past two years, or that has required the use of oral or parenteral corticosteroids at any time during the past two years)
    - Immunodeficiency disorder
    - Asplenia or functional asplenia
    - Diabetes
    - Deep venous thrombosis or thromboembolic event
    - Seizures (exception is simple febrile seizures during childhood)
11. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies
12. History of a severe allergic reaction or anaphylaxis following previous vaccinations or medications
13. Known allergies or other contraindications against: artemether-lumefantrine, PfSPZ Vaccine, or human serum albumin

14. Current or planned participation in an investigational vaccine study through the last required protocol visit.
15. Other condition(s) that, in the opinion of the investigator, would jeopardize the safety or rights of a participant participating in the trial, interfere with the evaluation of the study objectives, or would render the subject unable to comply with the protocol

6.3 INCLUSION OF VULNERABLE PARTICIPANTS

6.3.1 Participation of family members of study team members

Family members of study team members may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's family members employment.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable and unidentifiable manner.

6.4 INCLUSION OF PREGNANT WOMEN, FETUSES OR NEONATES

This trial will not include pregnant women, fetuses, or neonates as they are outside the targeted population age range.

6.5 LIFESTYLE CONSIDERATIONS

Not applicable.

6.6 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently enrolled in the study due to failure to satisfy eligibility criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not initially meet the criteria for participation in this trial (screen failure) because of an abnormal vital sign, laboratory, or ECG finding that may be transient (e.g., secondary to acute illness) may be rescreened at the principal investigator's (PI's) discretion. Rescreened participants will maintain the same participant number as for the initial screening.

6.7 STRATEGIES FOR RECRUITMENT AND RETENTION

6.7.1 Costs

There is no cost to subjects for taking part in this trial.

6.7.2 Compensation

Subjects will be given in kind (such as rice and/or millet) or cash equivalent, that can be given in multiple installments if preferred (e.g. for screening, at each vaccination visit, and periodically through the natural human malaria infection [NHMI] period), to compensate for the time taken to come to the study clinic for study-related visits. Preferred compensation will be decided in

consultation with village elders, but based on past studies in the community rice, millet, or cash have been acceptable.

The amount equals US $6 or US $3 for each visit depending on the time spent by the volunteer. Families will receive US $6 for visits with multiple procedures, venous blood draws, and longer study visits while US $3 for brief visits and limited blood draws and/or procedures. Unscheduled visits for clinical care only (no research sample collection or procedures) will not be compensated. Volunteer compensation payments will be made periodically throughout the study.

Table 11. Estimated compensation for the study.

Study Group	US Dollar Equivalent	Rice or Millet Dispensed or Cash equivalent (Local Currency [CFA][A]
All arms	$123	61,000 CFA

[A] Assuming currency exchange rate of US $1= 500 CFA; current (April 2021) conversion US $1 = 500 CFA.

7. STUDY INTERVENTION

7.1 STUDY INTERVENTIONS(S) ADMINISTRATION

7.1.1 Study Intervention Description

PfSPZ Vaccine

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

is dispensed into vials that are stored in liquid nitrogen vapor phase (LNVP) at -150°C to -196°C. All the procedures are described in detail in the cross-referenced IND .

Diluent

The diluent for PfSPZ Vaccine is composed of phosphate-buffered saline (PBS) and about 1% human serum albumin (HSA). HSA is a licensed product which is approved for parenteral, IV administration to humans and is purchased by Sanaria Inc. Diluent is released with a Certificate of Analysis (CoA), and is placed on a stability program. Vials of diluent will be shipped to the clinical site.

PBS that will be used was manufactured in compliance with GMP by Sanaria Inc. (9800 Medical Center Dr., Rockville, MD, USA). A Certificate of Analysis (CoA) is generated for each lot of PBS that is released for use in clinical studies. In addition, the PBS lots are placed on stability.

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

0.9% Sodium Chloride Normal Saline (NS) (Placebo)

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

Artemether Lumefantrine (AL)

Artemether-lumefantrine (AL) will be administered approximately 1-2 weeks prior to the first and perhaps third dose of PfSPZ Vaccine or normal saline. AL will be purchased from a licensed pharmacy in Mali from a single manufacturer and lot number.

7.1.2 **Dosing and Administration**

PfSPZ Vaccine + Diluent

The dose of PfSPZ Vaccine administered to vaccinees is $9x10^5$ PfSPZ in a range 0.25 to 0.5 mL of diluent. The dose of NS is likewise 0.5 mL. Each product is a clear, non-viscous liquid that has no perceptible color or odor. Each product is received by the blinded clinical vaccine administration team in syringes that appear identical in every way with labels and documents that ensure blinding of the clinical vaccine administration team but clear tracking of volunteers and syringes. Each is administered by DVI. PfSPZ Vaccine, diluent and NS will be shipped by Sanaria to the study site in Mali, where it will be received by Pharmaceutical Operations staff.

The syringes are prepared by the unblinded Pharmaceutical Operations team following SOPs. The preparation area is not accessible by clinical staff during syringe preparation or at any time when the unblinded randomization list is being reviewed. After preparation, the filled syringes are provided to the clinical team for DVI. The Pharmaceutical Operations team does not interact with study participants. The maximum allowable time between thawing of a PfSPZ Vaccine vial and injection is 30 minutes.

To perform DVI, a superficial vein in the arm or hand is located, prepped for venipuncture, a restricting band is placed on the arm, the needle is inserted into the vein, the plunger is

withdrawn slightly to cause blood flashback thereby confirming successful venous entry, the restricting band is removed, and the contents of the syringe are injected over a few seconds. Hemostasis is achieved by immediate direct pressure after withdrawing the needle. The syringe and needle are discarded into a sharps/biohazard container. The subject is then transferred to the observation area.

During administration of PfSPZ Vaccine, advanced-life-support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. The study staff administering PfSPZ Vaccine will wear gloves. The subject will stay in the clinical area for at least 30 minutes after vaccine administration.

0.9% sodium chloride Normal Saline (NS) (Placebo)

NS will be injected by needle and syringe into a peripheral vein by a qualified member of the clinical team. NS will be prepared such that a defined volume will appear indistinguishable from PfSPZ Vaccine.

During administration of NS, advanced-life-support drugs, trained personnel, and resuscitation equipment will be immediately available for the management of anaphylaxis. The study staff administering NS will wear gloves. The subject will stay in the clinical area for at least 30 minutes after vaccine administration.

Artemether Lumefantrine

All antimalarial medications used for the study will be maintained at the study site and administered by direct observational therapy (first dose) by a study team member. AL (and other antimalarials) will be purchased from commercial sources as detailed above and provided by the MRTC study team to subjects for dosing around vaccination only.

7.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

7.2.1 Acquisition and Accountability.

PfSPZ Vaccine

PfSPZ Vaccine is manufactured and released for use by Sanaria Inc. Sanaria will supply the investigational product, diluent and NS placebo to the clinical site. Syringes will also be supplied by Sanaria Inc. The pharmacist at the study site will ensure that all investigational products are inventoried and stored as indicated in the relevant SOP. Accurate records will be maintained regarding receipt of PfSPZ Vaccine, diluent and NS, as well as syringes which include: date received, lot number, amount received and disposition.

Sanaria will receive copies of document records in parallel regarding disposition of product, which include:

- Participant identification number
- Date and time PfSPZ Vaccine vial is thawed and time PfSPZ Vaccine is inoculated.
- Volume injected
- Lot number
- Signature of the person preparing the syringe
- Signature of the verifier
- Signature of the person administering the injection

- Signature of the verifier

Any partly used vials of PfSPZ Vaccine, NS, as well as empty vials, will be destroyed according to the applicable SOP and as determined by Sanaria. An inventory of the number of vials shipped versus used will be recorded. Unthawed and unopened vials of PfSPZ Vaccine and unused and unopened diluent vials and NS vials will be inventoried and returned to Sanaria.

PfSPZ Vaccine is not a licensed product and must be distributed under an Investigational New Drug Application (IND) in accordance with FDA regulations. The product must be administered by or under the supervision of the principal investigator or a subinvestigator of the clinical study, with the exception of syringe preparation which is delegated to the Pharmaceutical Operations team. Sanaria personnel will provide oversight of the syringe preparation work performed by the Pharmaceutical Operations team.

The clinical site must confirm that the vials of PfSPZ Vaccine have been transported and stored below -150°C according to SOP. Immediately prior to use, the cryovials will be thawed by trained Pharmaceutical Operations staff.

All PfSPZ Vaccine vials that are used will be documented on inventory forms as well as documented disposition forms according to SOP. All unused PfSPZ Vaccine vials will be returned to Sanaria Inc.

Diluent

The Diluent is phosphate buffered saline (PBS) containing human serum albumin (HSA). Diluent will be provided to the clinical sites by Sanaria Inc. The clinical site must confirm and document that the vials of diluent have been transported and stored within specified ranges. All diluent vials that are used will be documented on inventory forms as well as documented disposition forms according to SOP.

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

0.9% Sodium Chloride Normal Saline (NS) (Placebo)

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

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. All unused NS vials will be returned to Sanaria Inc.

Artemether Lumefantrine

Dosing of artemether lumefantrine will be used according to the package label.

7.2.2 **Formulation, Appearance, Packaging, and Labeling**

PfSPZ Vaccine

PfSPZ Vaccine is a suspension of aseptic, purified, metabolically active, non-replicating (live, radiation attenuated) cryopreserved Pf sporozoites (PfSPZ) formulated in cryoprotectant and

dispensed in vials. PfSPZ Vaccine is stored in liquid nitrogen vapor phase (LNVP) at -150°C to -196°C, thawed, diluted in diluent and administered by DVI.

Each cryovial is labeled indicating that it contains PfSPZ Vaccine, together with the lot number and date of manufacture.

The label for PfSPZ Vaccine reads as follows:

Label Name	Actual Size	Enlarged Size
Lot#XXXXXX	PfSPZ (NF54) VACCINE SANARIA® Lot#XXXXXX Manufactured: MMM YYYY SINGLE DOSE VIAL 900,000 SPZ in 0.02 mL Store in liquid nitrogen vapor phase Caution: New Vaccine Ltd by Federal Law to Investigational Use Only	**PfSPZ (NF54) VACCINE SANARIA®** **Lot#XXXXXX Manufactured: MMM YYYY** **SINGLE DOSE VIAL 900,000 SPZ in 0.02 mL** **Store in liquid nitrogen vapor phase** **Caution: New Vaccine Ltd by Federal Law to Investigational Use Only**

0.9% Sodium Chloride Normal Saline (NS) (Placebo)

The NS to be used in this trial will be an FDA licensed product which is commercially available (0.9% sodium chloride for injection 10 mL by Pfizer[4]). NS will be purchased by Sanaria and supplied to the clinical site.

Artemether Lumefantrine

The artemether lumefantrine used in this trial will be an FDA licensed product which is commercially available, purchased from a licensed pharmacy in Mali, and used according to the package label.

7.2.3 Product Storage and Stability

PfSPZ Vaccine

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

Transfer, receipt and maintenance of PfSPZ Vaccine from its storage site to the clinical trial site will follow SOP331 (Cryopreserved Material Transportation), provided by Sanaria Inc. At the study site, the LNVP shipper will be continuously monitored by a data logger as well as a

[4] https://www.medline.com/product/Sodium-Chloride-For-Injection-10ml-by-Pfizer/Z05-PF30165

temperature probe according to Sanaria standard operating procedure (SOP). Receipt of the products will be documented on a tracking log by trained study staff according to Sanaria SOP.

Diluent

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

0.9% Sodium Chloride Normal Saline (NS) (Placebo)

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 if used in a biological safety cabinet to maintain asepticity over a period of a few hours (i.e. the duration of vaccine preparation on a given day).

Artemether Lumefantrine

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

7.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

7.3.1 Randomization

Randomization will occur in blocks at 1:1 into vaccine or placebo arms. Randomization will be assigned at the time of first artemether/lumefantrine dose with the next available subject. Once a subject has received their first vaccination, they cannot be replaced.

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

If a subject is given the wrong treatment at V1 due to an allocation error, the same vaccine will be administered at V2 and V3 so long as the error is identified before the timing of the next injection. In the situation where an eligible participant already assigned a randomization number becomes ineligible during the window between randomization number assignment and administration of V1 (e.g., as a result of new information about the participant, withdrawal from participation, or other factors), a replacement will not be assigned, and the randomization number will not be assigned to another participant. This occurrence is anticipated to be a rare event.

7.3.2 Blinding

The study is double-blind (clinical staff and participants). Blinding extends to the parents and/or guardians of pediatric participants, to laboratory staff conducting clinical chemistry, hematology, and parasitology tests, and to laboratory staff conducting research assays such as antibody and cellular immunity assays. Blinding will continue until completion of the last study visit and the cleaning and locking of the data set. The principal investigator will be responsible for strict maintenance of the blinding on site.

7.3.3 **Unblinding**

Details of unblinding procedures are provided in the unblinding SOP. An overview is provided here.

Unblinding of individual participants: If knowledge of the treatment assignment is needed to provide appropriate medical care, and unblinding is recommended by the principal investigator, the local safety monitor (LSM), or the DSMB, the treatment assignment of that research subject may be unblinded and provided to the treating clinician and other clinical staff on a need-to-know basis by the head of Pharmaceutical Operations at the study site or other designated unblinded staff who have access to the unblinded randomization list and pharmaceutical team records. The principal investigator must contact the Sponsor and provide documentation of the event and the reasons for unblinding.

If pausing criteria are met based on blinded data, the following procedure will be followed, as described in **Section 7.1.1**. The principal investigator (who remains blinded) will provide the list of research subjects contributing to pausing criteria to the head of Pharmaceutical Operations or their designee, and will also inform the head of Pharmaceutical Operations how many of these research subjects have to be vaccine recipients (as opposed to NS recipients) in order to know if pausing criteria have truly been met. The head of Pharmaceutical Operations will then privately identify the treatment assignment of each participant on the list, and then inform the principal investigator if the pausing criteria have been met, but will not unblind the principal investigator with respect to individual participants.

7.4 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

8. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

8.1 SUSPENSION OF STUDY INTERVENTION

In this section, study "pause" indicates that immunizations will be briefly stopped for a particular group to allow consultation with the Sponsor and DSMB. Immunizations may resume immediately based on (1) the decision of the Sponsor, taking into account any recommendations of the DSMB and (2) concurrence by the principal investigator. IRBs and regulatory authorities will be notified but need not be involved in the decisions. Alternatively, if a study has been paused, the Sponsor may decide to impose a "safety hold," implying the need for wider consultation, potentially including IRBs and regulatory agencies, depending on the circumstances. A pause or a safety hold may be restricted to a particular subgroup, depending on the circumstances.

The study intervention will be paused if specified criteria in **Section 7.1.1** are met (see below). Discontinuing vaccinations does not mean discontinuation of study visits or procedures related to safety. Study procedures related to research assays, such as collecting samples for immunology tests, may be continued if participants and their parents/guardians agree.

7.1.1 **Pausing Criteria, Unblinding and Safety Hold**

As described in Background and Risk sections above, PfSPZ Vaccine has been safe and well tolerated in three randomized, double-blind, normal saline placebo-controlled trials in African children of the same or similar ages as planned for enrollment in this trial: BSPZV2 in Tanzania (3), KSPZV1 (Part 1) in Kenya (31), and EGSPZV2 in Equatorial Guinea (Jongo et al, unpublished data). In these trials, there was no difference in the rate or severity or nature of AEs or laboratory abnormalities compared to the normal saline controls. PfSPZ Vaccine also appeared to be safe and well tolerated in a fourth trial in 1-to-12 year olds underway in Lambaréné Gabon based on blinded data (LaSPZV1). Finally, it was safe and well tolerated in a large trial of Kenyan infants, KSPZV1 (Part 2), where approximately 80 infants received three doses of $1.8x10^6$ PfSPZ at 8 week intervals (Oneko et al, submitted) – twice the dose planned for 7-to-10-year-olds in the current trial –and where similar numbers of infants received three doses of $9.0x10^5$ or $4.5x10^5$ PfSPZ. Thus, it appears unlikely that safety issues will arise in the current trial. One note of caution, however, is that this will be only the second study where children have been administered a multi-dose priming regimen – two doses one week apart – the first being the Lambaréné trial, which enrolled about 128 children to receive PfSPZ Vaccine. It will therefore be important to continue careful recording of adverse events and to check safety labs after the second dose of vaccine.

Our pausing rules are established against this background. Since no adverse events are expected to occur at a rate higher than the background rate in the population, a small cluster of adverse events in vaccinees could signal a safety issue. For this reason, we have established the following two criteria. If either criterion is met, the clinical team will contact the Sponsor, which will contact the DSMB to review the events in question:

- Five percent or more of the vaccinees enrolled at any time point in one arm (calculated as 5% of the vaccinees in the full study roster enrolled to date, as long as there are at least three subjects with the AE) experience the same severe (grade 3) AE during the periods of AE collection deemed possibly, probably or definitely related to study product administration (PfSPZ Vaccine or NS), or experience the same grade 3 laboratory abnormality on day V2 + 14 or day V3+14. Grade 3 AEs deemed possibly, probably or definitely related to malaria (as opposed to PfSPZ Vaccine or to NS) will not count toward meeting this criterion. The site, where clinical staff are blinded, can only monitor total populations (vaccine plus placebo recipients) and if a 5% threshold is reached, unblinded study staff (e.g., the head of Pharmaceutical Operations) will need to confirm that the total indeed constitutes 5% of vaccinees. Note that we have considered using Grade 2 rather than Grade 3 laboratory abnormalities, but drops in hemoglobin, neutrophils, platelets and sometimes other blood count indices meeting Grade 2 criteria for severity are common in African pediatric populations.
- One participant experiences an SAE deemed possibly, probably or definitely related to study product. This criterion will not be met if it turns out that the participant received NS. SAEs related to malaria or to antimalarial treatment which are expected, will be reported expeditiously but will not fulfill this criterion. If the SAE is unexpected with respect to malaria or antimalarial treatment, it will fulfill the criterion.

If either of these criteria appear to be met by ongoing surveillance at the site, the principal investigator will engage the head of Pharmaceutical Operations on site, or his/her designee, to

follow unblinding procedures (see **Section 6.3.3** for a description). To meet the criteria, the subjects must actually have received PfSPZ Vaccine and not NS placebo.

After confirmation that a pausing criterion has been met, the Sponsor will contact the DSMB to describe the circumstances of the event(s). After an email discussion or, if recommended by the DSMB, an ad hoc teleconference, the DSMB, which will include the local safety monitor as a voting member, will make a written recommendation about whether or not the study should be unpaused, remain paused pending more data and further review, or placed on full safety hold, and whether or not the affected participants or their caregivers should be unblinded and notified of their treatment assignment. Based on the DSMB recommendation, a decision will be made by the Sponsor and principal investigator to either unpause the study and continue administration of investigational product in the affected group, to pause the study temporarily in the affected group to allow collection of more data about the triggering event(s) or other safety data, or to institute a full safety hold for the affected group or groups. IRBs (excepting IRBs providing administrative review) and regulatory authorities will be promptly informed of a study pause or of a full safety hold. Their permission will not be required to un-pause the study, but will be to reverse a safety hold.

Irrespective of the pausing criteria, the Sponsor, local safety monitor, principal investigator or applicable ethical committees or regulatory agencies may decide to pause the study, or to put the study on safety hold based on any constellation of AEs even if the 5% threshold is not reached and even if the AEs do not reach Grade 3 severity, or based on any other legitimate reason, such as AEs occurring in another study, product quarantine, manufacturing issues, expiry of permissions, out-of-specification stability test results, etc. For a full safety hold to be lifted, there must be written concurrence by the entity or entities that initiated the hold that the study can resume, and this may include regulatory agencies in addition to the two IRBs of record.

7.1.2 Temporary Hold of Vaccination of Individual Participants

The administration of PfSPZ Vaccine or NS to a study participant may be temporarily put on hold, based on clinical judgement, for the following reasons:

1. The participant is ill on the day of vaccination (administration may proceed in the case of a minor illness, based on the investigator's clinical judgment).
2. The participant has an abnormal laboratory value determined to be clinically significant and meriting delay in immunization, based on the investigator’s clinical judgment.
3. The participant has malaria or has taken medication for malaria or for another acute illness that may affect response to PfSPZ Vaccine. Participants with malaria will be treated with an antimalarial medication and administration of PfSPZ Vaccine will be postponed for a period of time after termination of treatment that is appropriate to allow sufficient washout of the drug.
4. The participant has received a non-live vaccine during the previous 14 days or a live vaccine during the previous 28 days (other than the investigational product) or three or more of any type of vaccine in the preceding four months.
5. At the request of the participant.

6. On the advice of the principal investigator, local safety monitor, IRB, regulatory authority, DSMB or sponsor.
7. On the advice of one of the investigators, as long as the principal investigator concurs.

Vaccination will be rescheduled at the earliest possible time within the acceptable window of the originally scheduled vaccination, or outside that window if there is no other option.

8.2 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from receipt of study products (AL, PfSPZ Vaccine or normal saline) for an individual does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include continued study procedures as outlined in the protocol. If no further vaccinations are to be administered, safety labs related to vaccination schedules may be deferred at the investigator's discretion.

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Completion of study intervention
- New diagnosis which requires discontinuation of the study intervention
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Investigator discretion
- Participant unable to receive AL dosing or subsequent vaccinations more than 7 days from proposed study window.
- Significant study intervention non-compliance
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject has completed the study follow-up period
- Death
- Screen Failure

8.3 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

The reason for participant discontinuation or withdrawal from the study will be recorded on the End of Study Case Report Form (CRF). Replacement of a subject may occur in the case that a parent/guardian signs the informed consent and the subject is randomized but the subject does not go on to receive the study intervention. In the case where a parent guardian/signs the informed consent, the subject is randomized, receives the first dose of PfSPZ Vaccine and the

subject is withdrawn or the subject or parent/guardian decides to withdraw, no subject replacement may occur.

8.4 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for scheduled visits and the parent/guardian is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the parent/guardian and reschedule the missed visit within the specified visit window and counsel the parent/guardian on the importance of maintaining the assigned visit schedule and ascertain if the parent/guardian (and if applicable, the subject) wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the parent/guardian (where possible, 3 telephone calls and, if necessary, a home visit to the participant's last known address). These contact attempts should be documented in the participant's study file.
- Should the parent/guardian continue to be unreachable, he or she will be considered to have withdrawn the subject from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

9.1 SCREENING PROCEDURES

9.1.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with the parents/guardians of prospective subjects.
- Review of existing medical records.

9.1.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the parent/guardian of the subject has signed the consent form for this study. Assessments performed at outside facilities or on another MRTC or NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

All screening tests and procedures must be performed within 56 days prior to enrollment (AL dosing), unless a time period is specifically mentioned.

- Malaria comprehension exam completed by the parent/guardian, wrong questions reviewed, and confirmed passing of ≥80%
- Demographic information will be obtained from interview of the subject's parent/guardian
- Complete past medical history will be obtained by interview of subjects' parent/guardian
- Eligibility criteria will be reviewed with subjects' parent/guardian

- All concomitant medications and vaccinations taken within one month prior to screening will be reviewed.
- Planned vaccinations and expected timing
- Result of laboratory tests will be reviewed and recorded
- Complete physical exam will be completed
- Vital signs will be obtained, including height, weight, temperature, heart rate, and blood pressure
- Blood collected for baseline laboratory evaluation, including malaria assessment
- Electrocardiogram completed

9.2 Enrollment and Artemether Lumefantrine Visit

- Prior to enrollment, all screening procedures will be confirmed completed
- Confirmation of the willingness to participate of the subject and the subject's parent/guardian
- Interim clinical (focused physical exam, medical) history will be obtained with updates on any changes since previous clinic visit or contact
- Eligibility criteria will be reviewed and confirmed by study PI
- Blood draw for research and clinical labs, including malaria assessment
- AL dosing started (first dose in clinic by directly observed administration)

9.3 Vaccination Visits

- Confirmation of the willingness to participate of the subject and the subject's parent/guardian
- Interim clinical (focused physical exam, medical) history will be obtained with updates on any changes since previous clinic visit or contact
- Eligibility criteria will be reviewed and confirmed by study PI
- Blood draw for research and clinical labs, including malaria assessment
- Vaccination completed
- Post vaccination observations and evaluation completed

9.4 Safety Follow-up Visits

- Confirmation of parent/guardian of subject's continued willingness to participate
- Interim clinical (focused physical exam, medical) history will be obtained with updates on any changes since previous clinic visit or contact
- Blood draw for research and clinical labs, including malaria assessment
- Solicited local and systemic reactogenicity completed (through 7 days post vaccination)

9.5 Efficacy Assessments

- Confirmation of parent/guardian of subject's continued willingness to participate
- Interim clinical (focused physical exam, medical) history will be obtained with updates on any changes since previous clinic visit or contact
- Blood draw for research and clinical labs, including malaria assessment

9.6 UNSCHEDULED VISITS

- Interim clinical (focused physical exam, medical) history will be obtained with updates on any changes since previous clinic visit or contact
- Blood draw for clinical labs, as indicated, and research labs (with unscheduled blood smear positive visit)

9.7 CLINICAL EVALUATIONS

Complete physical examination: (e.g., height and weight, heart rate, organ systems, or other functional abilities).

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

Malaria blood smears: The gold standard for malaria diagnosis and evaluation of VE endpoints is the detection of malaria parasites on Giemsa-stained thick blood films. Blood smears are prepared in duplicate according to standard malaria challenge procedures and evaluated by trained study microscopists, and the results reported to the study PI. At least 0.5 µL are scanned for the presence of malaria parasites. This method allows for detection of a parasite density of approximately 2 parasites/µL and early diagnosis, often before subjects become symptomatic for malaria. Slides are considered positive if at least one unambiguous *P. falciparum* parasite per slide are identified and confirmed by a second microscopist.

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

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

Passive and active case detection: Contact information for the study clinic and principal investigator will be provided to the parent/legal guardian of all participants so they will be able to easily schedule a clinical evaluation for any symptoms of concern experienced by the study participant. Study participants are also welcome as walk-ins at the study clinic. Parents or guardians will be actively encouraged throughout the trial to report to the clinic whenever a participant is ill.

In addition, active outreach to study subjects will occur no less frequently than every two weeks during the malaria surveillance period. Parents / participants will be questioned at these visits for history of malaria symptoms including subjective fever, and body temperature will be assessed at each encounter (see Table 13).

Blood smears will be collected and examined in the case of any participant presenting with signs or symptoms consistent with malaria. Blood smears will additionally be collected every four weeks. However, the results of blood smears collected from asymptomatic participants will be reported in the clinical database after surveillance is complete so as not to interfere with the detection of malaria *with symptoms* (clinical malaria episodes).

A short questionnaire about bednet use will be filled out at each clinic visit to document this variable, that could affect study results.

9.8 UNSCHEDULED BLOOD SMEAR POSITIVE VISITS

After receipt of at least one vaccination, if a subject has detected *Pf* malaria parasites on peripheral thick blood smear (scheduled or unscheduled visits), they will be asked to the clinic to provide an additional blood sample within 48 hours for the following:

- 0.5 mL EDTA microtainer for whole blood ex-vivo assays **if** sample not already obtained within the last two days (i.e. at the time of the positive blood smear)
- 4 mL EDTA tube to obtain RNA and DNA for the study of host and parasite transcriptome (RNA) and parasite genotype (DNA) and to obtain plasma for proteomics studies
    - If 4mL EDTA sample has been collected in the last 28 days for this purpose, it can be deferred at the investigator's or subject's discretion.
- 0.5 mL for qPCR **if** sample not obtained within the last two days with the positive blood smear

N.B.: scheduled smears on asymptomatic during the NHMI phase will be read and/or reported retrospectively and smear positive visits will not apply

The unscheduled blood smear positive blood draw does not need to be completed with every positive blood smear and subjects have the right, as with every blood draw, to refuse to return for this additional blood draw. While subjects may be asked back to the clinic after at least one vaccination, preference for unscheduled blood smear positive blood draws are for first positive blood smear post vaccination #3, subsequent positive blood smear after treatment with anti-malarial treatment (>14 days after treatment), and new positive blood smear after having a negative blood smear >28 days. If repeat blood smears are done to monitor the clearance of parasitemia following treatment, these additional labs will not be drawn.

9.9 MALARIA QPCR

While detection of parasites on thick blood smears remains the most common primary endpoint in human challenge trials, both PCR- and nucleic acid sequence-based amplification (NASBA)-based methods have been increasingly used to support blood smear data in malaria vaccine trials (32, 33). These research molecular assays have significantly increased sensitivity for detection of *P. falciparum* blood-stage infection approaching 20 parasites/mL, often resulting in diagnosis 2-4 days earlier than by paired thick blood smears (32, 34, 35). 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 may be performed from all scheduled visits with a malaria blood smear noted to capture infections that remain below the detection limit for microscopy. For subject convenience, a

finger prick sample can be used for both preparation of the microscopy slide and for DNA preservation.

In the case of positive blood smears that constitute a primary or secondary endpoint for a participant, an FDA qualified qPCR assay will be performed by the laboratory of Dr. Sean Murphy, University of Washington, as confirmatory.

Immunological Assays

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

These assays include:

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

Sanaria may also assess antibodies to whole PfSPZ by immunofluorescence assay (IFA) and inhibition of sporozoite invasion assay (ISI) and to asexual erythrocytic stage parasites by IFA as described (36, 37).

Laboratory assays to assess immune responses to novel pre-erythrocytic antigens may be performed in the laboratories at the LMIV, NIAID, and NIH according to standard laboratory procedures. The target proteins are novel antigens that confer protection against liver stage malaria in rodent malaria models (*P. yoelii*, *P. berghei*) according to vaccination studies conducted by Seattle BioMed (part of Seattle Children's Research Institute) and LMIV. The novel antigens to be used for these laboratory assays include PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113, using the identifiers in the PlasmoDB database (www.plasmodb.org). These antigens were initially selected on the basis of their gene expression during early liver stage development of *Pf*, and preliminary testing shows that these antigens are immunologically recognized by individuals previously exposed to *Pf*. 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 Vaccine 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.

9.9.1 Samples for Genetic/Genomic Analysis

Description of the scope of genetic/genomic analysis

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

Description of how privacy and confidentiality of medical information/biological specimens will be maximized

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

9.10 Safety and Other Assessments

9.11 Adverse Events and Serious Adverse Events

9.11.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

9.11.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of

the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

9.11.3 **Classification of an Adverse Event**

Severity of Event

For adverse events (AEs) not included in the protocol defined grading system (see Appendix A), the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship

improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

Expectedness

Principal investigators or designees will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

9.11.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study after enrollment, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All solicited (see **Table 12** below) and unsolicited AEs will be recorded through Day 7 after each injection. Injection site reactions will be assessed until Day 7 after injection (PfSPZ Vaccine or normal saline) or until resolved.

After the periods specified above only unsolicited aEs, SAEs, UPs, and new onset of chronic illness (NOCI) will be recorded.

Table 12. Solicited Adverse Events

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

Generalized pruritus	Generalized Edema
Drowsiness	Fever or feverish
Inability/refusal to eat or drink	Irritability/fussiness
Nausea/Vomiting	Diarrhea
Symptoms to be solicited during malaria monitoring phase – study days 43 through 211	
Headache	Chills/rigors
Malaise/fatigue	Dizziness/lightheadedness
Myalgias/arthalgias	Fever or feverish

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

Any laboratory abnormalities (other than those specified as safety labs in the protocol as defined by the values in the toxicity table) should be reported as AEs if they require intervention. Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments, or concomitant treatment. In addition, any medically important laboratory abnormality may be reported as an adverse event at the discretion of the investigator. This could include a laboratory result for which there is no intervention, but the abnormal value suggests a disease or organ toxicity. In addition, similar to solicited AEs, all laboratory AEs will be collected and graded for severity through 7 days after each vaccination until resolved.

The Investigator will assess all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following sections in the protocol.

9.11.5 Adverse Event Reporting

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.

9.11.6 Serious Adverse Event Reporting

The study investigator will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting

documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

All SAEs (regardless of relationship and whether or not they are also UPs) will be reported and sent to the IND sponsor by fax (SAE fax line: 240-306-0596) or email attachment. Deaths, immediately life threatening and all possibly, probably or definitely related SAEs will be communicated by telephone, fax, email or automated report via the data management system by the PI **within 24 hours** of site awareness of occurrence to the IND sponsor. All other SAEs will be reported to the IND sponsor **within three business days** after the site becomes aware of the event. The PI must document that the communication is received and acknowledged.

Sanaria Inc.

SAE Fax: 240-306-0596

Individuals:

1. Stephen L. Hoffman, M.D.

Email: slhoffman@sanaria.com

2. Thomas L Richie, M.D., Ph.D.

Email: trichie@sanaria.com

3. L.W. Preston Church, M.D.

Email : lwpchurch@sanaria.com

4. Tooba Murshedkar, MS
SAE Fax: 240-306-0596

Email: tmurshedkar@sanaria.com

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

LMIV Contact Information:

PI: Patrick Duffy, MD

Tel: 301-761-5089

Fax: 301-480-1962

Email: patrick.duffy@nih.gov

AI: David Cook, MD

Tel: 240- 627-3066

Fax: 301-82 7-1661

Email: david.cook@nih.gov

Independent Safety Monitor:
Prof. Abdoul Aziz Diakite, MD
Head, Department of General Pediatrics
Gabriel Touré University Hospital
Bamako, Mali
Phone : + 223 66 74 49 56

Email: doc_abdela@yahoo.fr

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

9.11.7 Events of Special Interest

Seizures of any severity will be considered an event of special interest and will be reported to the IND sponsor and FMPOS on the NIH Reportable Events Form sent by fax or email attachment no later than 7 calendar days of site awareness of the event.

All UPs that are also adverse events will be reported to the IND sponsor on the NIH Reportable Events Form sent by fax or email attachment no later than 7 calendar days of site awareness of the event.

UPs that are not AEs will also be reported to the IND sponsor.

9.11.8 Reporting of Pregnancy

Not applicable given all enrolled children will not be of childbearing potential.

9.12 Unanticipated Problems

9.12.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review

Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which may include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

9.12.2 Reporting Procedures to the FMPOS EC

The USTTB PI is responsible for reporting per FMPOS EC reporting requirements. All UPs, major protocol deviations, all non-compliance, new information that might affect the willingness of a subject to enroll or remain in the study, and any suspension or termination of research activity will be reported to the FMPOS EC within 7 calendar days of investigator awareness. Any death of a research subject that is possibly, probably, or definitely related to the research will be reported within 24 hours of investigator awareness.

The following events will be reported to the FMPOS EC as a high-level summary at the time of continuing review:

- AEs and SAEs that are not UPs, as a narrative summary indicating whether these events were within the expected range.
- Minor and major protocol deviations.
- UPs reported to the FMPOS EC.
- Non-compliance reported to the FMPOS EC that is not related to a protocol deviation.

10. STATISTICAL CONSIDERATIONS

This section describes the statistical analyses of primary and secondary study objectives. Further details, including methods for assessing exploratory objectives, are described in a separate statistical analysis plan to be generated prior to unblinding. Any changes to analyses described in the protocol or statistical analysis plan will be fully disclosed in the clinical study report.

10.1 STATISTICAL HYPOTHESIS

Primary Endpoints

Safety: The primary safety endpoint is the proportion of vaccinees experiencing related SAEs from V1 to 26 weeks after V3. There is no formal hypothesis test associated with this primary safety endpoint. Rather, the proportion of subjects experiencing related SAEs will be presented for each arm separately using frequencies, percentages, and descriptive 95% confidence intervals (CIs).

Efficacy: the primary efficacy endpoint is the vaccine efficacy against *malaria with symptoms.* For this protocol, there is a primary and a secondary case definition of Pf *malaria with symptoms*.

The primary case definition is as follows:

Pf *malaria with symptoms* is defined as a positive thick blood smear at a denisity of >1000 P/uL plus:

- Measured axillary temperature ≥ 37.5 degrees Celsius or history of fever (subjective or objective) in the last 24 hours, or,
- Symptoms of malaria –
    - Verbal individual (individual able and willing to answer questions): A verbal individual is considered symptomatic if reporting at the time of evaluation at least two of the following symptoms/symptom groups: headache, chills and/or rigors, malaise and/or fatigue, dizziness and/or light-headedness, myalgias and/or arthralgias; or
    - Non-verbal individual (small child or any individual unable or unwilling to answer questions): A non-verbal individual is considered symptomatic if manifesting at the time of evaluation at least two of the following signs/sign groups: drowsiness, irritability and/or fussiness, inability and/or refusal to eat or drink, prostration; or
- Any individual: Signs of severe malaria (e.g. impairment of consciousness, severe anemia, hemoglobinuria, acute kidney injury, etc.).

The secondary case definition is as follows:

Pf *malaria with symptoms* is defined as a positive thick blood smear at a density of > 0 P/uL plus:

- Measured axillary temperature ≥ 37.5 degrees Celsius or history of fever (subjective or objective) in the last 24 hours, or,
- Symptoms of malaria as defined in the primary case definition; or
- Meeting criteria for severe malaria

The formal hypothesis associated with the primary efficacy endpoint is a superiority test of

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

(equivalently, H_0: HR = 1 vs. H_A: HR <> 1). The primary efficacy hypothesis test will be conducted at the two-sided α=0.05 significance level, restricted to subjects in the mITT population (defined below in **Section 9.3**).

The primary efficacy endpoint will be assessed over all participants, the vaccinated versus controls.

Secondary Endpoints

*Safety***:** The secondary safety endpoints include the related SAEs as well as all other measures of safety and tolerability – unsolicited AEs, laboratory abnormalities, and solicited AEs.

Efficacy: The secondary efficacy endpoint is the vaccine efficacy against Pf *malaria*.

Pf *malaria* is defined as:

- At least one unambiguous asexual parasite on thick blood smear identified by two independent microscopists after each examining 0.50 µL of blood in a study participant

10.2 SAMPLE SIZE DETERMINATION

Power calculations for safety analysis:

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, within an arm of size n=120, there is a 90% chance of observing no event if the true rate is no more than 0.08%, and a 90% chance of observing at least 1 event if the true rate is no more than 1.9%.

Probabilities of observing 0, 1, and 2 or more events within an arm of size 120 are presented in **Table 13**, 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.

Table 13. Probability of observing 0, 1 and 2 or more events, within an arm of size 120 for different true event rates.

True event rate (%)	Pr(0/120)	Pr(1/120)	Pr(2+/120)
1	0.299	0.363	0.338
3	0.026	0.096	0.878
5	0.002	0.013	0.984
10	<0.001	<0.001	>0.999

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. **Table 14** shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. If none of the 120 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 0.030.

Table 14. Two-sided 95% confidence intervals based on observing a particular rate of safety endpoint arms for an arm of size 120.

Observed event rate	95% Confidence interval (%)
0/120	(0.000, 0.030)
1/120	(0.000, 0.046)
2/120	(0.002, 0.059)
3/120	(0.005, 0.071)
4/120	(0.009, 0.083)
5/120	(0.014, 0.095)
6/120	(0.019, 0.106)

The sample size determination is mainly based on the assessment of efficacy, which will be shown below.

Power calculations for efficacy analysis:

The primary efficacy endpoint is the efficacy against Pf *malaria with symptoms*. The vaccine efficacy will be assessed by comparing the vaccinated versus the controls.

Based on recent data from Bancoumana, the symptomatic malaria rate under the control is very likely above 0.35 for children of age 6-10. We estimate a sample size of 120 per arm will allow reasonable power to detect the between-arm difference.

Table 15 presents the power calculation for detecting vaccine efficacy based on a two-sided log-rank test with the familywise type I error of 0.05. In the power calculation, vaccine efficacy is 1 minus the probability of Pf *malaria with symptoms* among the vaccinated divided by the probability of Pf *malaria with symptoms* among the controls. A drop-out rate of 10% is assumed.

With 240 subjects in the two arms, this study has over 80% power to detect the vaccine efficacy if the Pf *malaria with symptoms* rate is no less than 0.35 under the control and the vaccine efficacy is no less than 0.5.

In order to allow a booster study to proceed during a second transmission season if VE is 50% or greater in the first year, an additional 10% dropout rate has been assumed and 14 participants added for a total sample size of approximately 134 in each arm in the first year.

Table 15. Power calculation for plausible Pf *malaria with symptom* rates for two-sided log-rank test under type 1 error rate of alpha=0.05.

Pf *malaria with symptoms* rate under control	Vaccine efficacy	Power(%) (90 per arm)	Power(%) (100 per arm)	Power(%) (110 per arm)	Power(%) (120 per arm)	Power(%) (130 per arm)	Power(%) (140 per arm)
0.3	0.4	43	47	50	54	57	60
	0.5	62	66	70	74	77	80
	0.6	79	83	86	89	91	93
0.35	0.4	50	55	59	62	66	69
	0.5	70	75	79	**82**	85	88
	0.6	86	89	92	94	95	97
0.4	0.4	58	63	67	71	74	77
	0.5	78	82	86	88	91	93
	0.6	91	94	96	97	98	98
0.5	0.4	73	78	81	85	87	90
	0.5	90	92	95	96	97	98
	0.6	97	98	99	99	100	100
0.6	0.4	86	89	92	94	96	97
	0.5	96	98	99	99	99	100
	0.6	99	100	100	100	100	100

10.3 POPULATIONS FOR ANALYSES

The following analysis populations will be used when describing subject disposition and performing statistical analyses:

- The **Screened Population** includes all subjects who are screened and provide informed consent, regardless of whether the subject is randomized or treated. This population will be used to fully account for subject disposition.

- The **Intention to Treat (ITT) Population** is a subset of the screened population that is deemed eligible to participate in the study, is randomized to a treatment group, receives *at least one injection* with PfSPZ Vaccine or placebo, and contributes evaluable person-time to the risk set.
- The **Modified Intention to Treat (mITT) Population** includes all subjects who are deemed eligible to participate, are randomized, receive *all three injections* of vaccine or placebo, and contribute evaluable person-time to the risk set, including subjects to whom V2 and/or V3 is administered out of the pre-specified time window or an incomplete injection is given, defined as an estimated injection volume of >10% and < 80%.
- The **According to Protocol (ATP) Population** is a subset of the mITT Population, including subjects who complete the vaccination series per protocol and contribute evaluable person-time to the risk set.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

Missing Data

Subjects who discontinue early or are lost to follow-up will be censored from applicable analyses at the last date where the study outcome can reliably be determined (e.g., date of last available TBS specimen without parasitemia).

It is anticipated that some event dates required for safety analyses may be incomplete. If the event onset day is unknown, then the date will be imputed as the last date the subject could reliably be assumed to be event free (e.g., the previous study visit where the event was not reported). If the resolution day is incomplete, then the date will be imputed as the date that the event was reliably known to no longer be present.

It is anticipated that some TBS results will be missing. In those instances where one TBS is missed, the result will be imputed to be the same as the result of the next TBS. Up to three such single missing TBS will be allowed in a research subject during the 26 weeks of follow-up without disqualifying the participant from the ATP population (as long as the individual meets other requirements for ATP, such as receiving all three immunizations within the correct time windows). If there are situations where two or more consecutive TBS are missed, the result will again be imputed to be the same as the result of the next TBS, and the research subject will be included in the m-ITT population (as long as the individual meets other requirements for m-ITT, such as receiving all three immunizations). If the only TBS missed is the last, there will be no imputation, the missing data point will be ignored, and the individual may be included in the ATP population. If the two or more TBS missed are the last, there will be no imputation, the missing data points will be ignored, and the individual may be included in the m-ITT population. However, if more than the last two TBS are missed, the individual will not be included in proportional efficacy analyses, although may be included in time-to-event efficacy analyses.

Multiple Testing

The primary VE endpoint is the VE against Pf *malaria with symptoms.* The overall vaccine efficacy will be assessed over all participants.

No multiplicity adjustment will be made for secondary efficacy or safety endpoints.

10.4.2 **Safety Analyses**

The primary safety endpoint is the occurrence of an SAE between V1 and 26 weeks after V3 which is at least possibly related to treatment. Serious AEs which meet this definition will be summarized in frequency tables and listings according to system organ class, preferred term, relatedness, and severity. The primary safety analysis will be performed using the ITT population and according to treatment received at V1, regardless of any randomization or allocation errors. All SAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized using frequencies, proportions, and exact 95% CIs for proportions. Rates of SAEs (overall and for any system organ class or preferred term with at least 5 events in either group) will be compared using two-sided 0.05 level Fisher's Exact Tests.

Secondary safety endpoints include the occurrence of unsolicited AEs, laboratory abnormalities, and solicited AEs in the ITT population. Unsolicited AEs documented from V1 to 14 days following V3 will be coded using MedDRA and summarized in frequency tables according to system organ class, preferred term, relatedness, and severity. Summaries will be provided overall and according to the vaccination number (1, 2, or 3) immediately prior to AE onset. Rates of unsolicited AEs (overall and for any system organ class or preferred term with at least 5 events in either group) will be compared using two-sided 0.05 level Fisher's Exact Tests. Adverse events leading to premature discontinuation from the study will be listed separately. Clinically relevant laboratory abnormalities recorded in the 14 days after V3 will be summarized in frequency tables by type, grade, and clinical relevance.

10.4.3 **Efficacy Analysis**

9.4.3.1 Primary: VE against Pf *malaria with symptoms*

The primary efficacy analysis will be the modified Intention to Treat (mITT).

The primary efficacy analysis will be based on time to the first Pf *malaria with symptoms*. Entry into the risk set begins on the latter of a) 14 days after completing the primary vaccination series (V3 + 14 days) or b) 28 days after initiating treatment for a parasitemia event that was detected on or before V3 + 14 days, whichever comes later. Time-to-event is defined as the number of days between entry into the risk set and date of first Pf *malaria with symptoms*; subjects who do not experience the event by 26 weeks after V3 will be censored from the primary analysis or earlier date of last negative TBS result.

The survival patterns will be described by Kaplan-Meier curves for each arm and compared by the logrank test between two arms. The protective efficacy will be assessed from the Cox proportional hazards model with treatment arm as the regressor, and the vaccine efficacy will be estimated as one minus the hazard ratio.

As a secondary efficacy analysis, the cumulative probability of Pf *malaria with symptoms* through 26 weeks post V3 will be presented for each treatment arm and compared across arms based on Kaplan-Meier estimates along with 95% confidence intervals. This estimation accounts for right censoring and the comparison should be equivalent to Fisher's exact test in case of no censoring.

Sensitivity analyses will include an ITT and ATP analysis with entry into the risk set as with the mITT analysis, and ATP analyses with entries into the risk set at time of first vaccination and at V3.

9.4.3.2 Secondary: VE against Pf *malaria*

VE against Pf *malaria* (see definition in **Section 9.1**) will be assessed as for VE against Pf *malaria with symptoms*, using the mITT population and censoring participants who do not experience the endpoint by 6 months after V3.

The cumulative probability of Pf *malaria* through 26 weeks post V3 will be similarly presented as for Pf *malaria with symptoms*.

10.4.4 **Exploratory Analyses**

The trend of the immune response, more specifically the humoral and cellular immune responses, to PfSPZ Vaccine will be modelled and compared between the vaccine recipients and the controls. This will be achieved via a generalized linear regression which accounts for the within-subject correlation.

To assess genetic relatedness of the PfSPZ Vaccine parasite strain to malaria infection parasites, a genotypic sieve analysis to analyze the sequences of peripheral blood *Pf* parasites from infected subjects. The sieve analysis targets at differentiating protective efficacy against different genotypes of infection-inducing parasites with genotype defined by the number of mismatches to the PfSPZ footprint.

10.4.5 **Planned Unblinded Analyses**

The study statistician will undertake an unblinded preliminary analysis of the safety and efficacy data from the trial after the data for all originally-planned primary and secondary endpoints have been gathered but before formal database lock. This will allow for the timely planning of future trials in this study population.

The results of these analyses will be transmitted by the study statistician to the LMIV Senior Investigator only. This will allow the remainder of the study team, including the PI, to remain blinded in the case of a future extension of the trial such as for a booster vaccination, extended follow-up, or a combination of both. The LMIV Senior Investigator may share the results of the unblinded analysis with the trial sponsor, Sanaria, the DSMB, and other unblinded parties as appropriate for the purposes of future planning.

The analyses of the Primary Objectives will include measurement of VE against first episode of Pf *malaria with symptoms* by time-to-event analysis.

The analyses of the Secondary Objectives will include measurement of VE against first episode of Pf *malaria* (parasitemia) with or without associated symptoms by time-to-event analysis.

Other unblinded analyses of safety and efficacy may also be requested by the Senior LMIV Investigator. For example, analyses of the Exploratory Objectives may include measurement of VE using other outcome measures such as against all episodes of Pf *malaria with symptoms* and Pf *malaria* rather than against the first episode, using proportional analysis, or using ITT or ATP populations, or using parasite density thresholds of > 5000 parasites/μL.

11. REGULATORY AND OPERATIONAL CONSIDERATIONS

11.1 INFORMED CONSENT PROCESS

11.1.1 Consent Procedures and Documentation

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 and their parents or guardians will be given the opportunity to ask questions and have them answered.

Participants and their parents or guardians will be asked to read and review the consent documents. The study staff member will explain the research study to the participant and answer any questions. A verbal explanation will be provided in terms suited to the participant's comprehension, and will include the purposes, procedures, and potential risks of the study. Participants will be informed of their rights, such as the fact that participation is voluntary and that they may withdraw from the study at any time, without explanation or prejudice.

After the verbal explanation, participants (and/or their parents or guardians) will have the opportunity to carefully review the written consent form and ask questions prior to signing. They will also have the opportunity to discuss the study with family members or others they may choose, or to request additional time to consider prior to agreeing to participate.

Assent, either written or verbal, is not typically obtained for minors under the age of 12 in Mali. Therefore, assent will not be documented on this trial enrolling only children under the age of 10. Nevertheless, consent will be obtained from participants' parent(s) and/or guardian(s) in a location that is supportive of children.

The participation of both parents in the consent process, and the signature of both parents will be encouraged.

The informed consent process will be conducted and documented on the source document by signature and date, before any study procedures can begin. A copy of the informed consent document will be given to the participants' parent(s) and/or guardian(s) for their records. Consent forms will be approved by all participating IRBs. The participants' parent(s) and/or guardian(s) may withdraw consent at any time throughout the course of the trial. The informed consent process will be documented in the subject's research chart, as required by 21 CFR 312.62. The ICF will be signed (or fingerprinted) and personally dated by the subject (or subject's legal guardian) and the person who conducted the informed consent discussion. The original signed ICF will be retained in the subject's chart and a signed and dated copy will be provided to the subject. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Community Permission in Mali

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

- Study investigators/personnel explain the study to village leaders, including the village chief, family heads, women's 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.

The individual informed consent process and relevant forms 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.

Malaria Comprehension Exam

Subjects or their parents/guardians will be encouraged to ask questions, and then the parents/guardians will take a multiple-choice questionnaire (true/false) to evaluate consent comprehension. All incorrect responses will be reviewed with the parents/guardians, and they must verbalize understanding of all incorrect responses. A score of ≥80% correct is required for enrollment. For parents/guardians scoring less than 80%, study staff may choose to review study details again with subject and parents/guardians and reassess comprehension with a repeat Malaria Comprehension Exam. At the discretion of the investigator, any subject or parent/guardian 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 parents/guardians 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 the parents/guardians. This will help ensure that the parents/guardians have sufficient understanding before the consent form is signed. The parents/guardians may either sign the consent form immediately or later after further consideration. parents/guardians 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.

11.1.2 **Consent for minors when they reach the age of majority**

We request a waiver of informed consent to continue to use data and/or specimens for those individuals who reach the age of majority after all study visits are complete. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate these subjects to complete the standard protocol consent. The retention of samples and data will not affect the welfare of these subjects.

11.1.3 Considerations for Consent of family members of study team members

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible.

11.1.4 Consent of Subjects who are, or become, decisionally impaired

Not applicable.

11.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the principal investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and, as applicable, the Food and Drug Administration (FDA).

11.3 Confidentiality and Privacy

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

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the MRTC, NIH, and Sanaria Inc. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the study team research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

11.4 FUTURE USE OF STORED SPECIMENS AND DATA

All samples stored for use in the trial will be labeled with the participant's study identification (ID) number, which cannot identify the study subject directly (not linkable to any personally identifiable information) but is linkable to other research databases (e.g., questionnaires, CRFs, logbooks) generated by the study. The subject ID log linking the study subject's ID number to the name of the participant will be kept secure, with access limited to authorized research team members.

At the completion of the clinical trial and the exploratory studies described in the protocol, samples may be destroyed, transferred to another subsequent trial, or stored, the latter only if permission has been given for future use and the IRBs are informed. If stored, they will be coded to allow linking with relevant clinical data without the possibility to link back to the study subject.

Samples may be used in the future to learn more about protection from malaria, vaccine-induced immunity and confounders of vaccine take. The data generated in the study will form the basis for further studies using these specimens aimed at defining the immune responses to Pf infection that lead to protection. For any studies that are not specifically established in the ICF, the investigators wishing to study these samples will need to obtain approval from the IRB.

Participants may withdraw permission for future use of specimens at any time. If a participant withdraws his or her permission for future use of specimens, the investigator or designee will destroy all known remaining specimens and report this destruction to the study subject and the IRBs and make a note in the study subject's chart. This decision will not affect the research subject's participation in this trial or any other trials that are overseen by the IRBs. If specimens for future use have already been de-identified, their destruction may not be possible.

Temperatures will be monitored continuously in freezers and refrigerators where samples are stored.

11.5 SAFETY OVERSIGHT

As agreed with the Office of Clinical Research Policy and Regulatory Operations (OCRPRO), a DSMB chartered by the IND sponsor, Sanaria Inc. will be used for this study. Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including malaria vaccine and pediatric experience. Members of the DSMB will be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest.

The DSMB will review the study prior to initiation, after completion of the vaccination series and at study close. The board may convene additional reviews as necessary, and will issue recommendations concerning continuation, modification, or termination of the study. All SAEs will be reported by the PI to the sponsor immediately upon becoming aware of them. All SAEs that are possibly, probably or definitely related to the study agent, UPs, and safety reports (as available) will be reported by the sponsor to the DSMB.

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

The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to Sanaria Inc.

11.6 CLINICAL MONITORING

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 and 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.

11.7 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

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

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

11.8 Data Handling and Record Keeping

11.8.1 Data Collection and Management Responsibilities

In Mali, study data will be entered directly into a study-specific DataFax electronic database. Data from electronic CRFs will be collected directly from subjects during study visits and telephone calls or will be abstracted from subjects' medical records. Electronic CRFs and supporting laboratory documentation will be used as source. Any type of corrections to the electronics CRFs will be documented and tracked. All CRFs should be reviewed by the investigator and signed as required with written signature.

Data entry will be performed by authorized individuals. Corrections to the electronic data systems will be tracked electronically (password protected or through an audit trail) with time, date, individual making the correction, and what was changed. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. NIH researchers will access coded data only, and will not have access to personally identifiable information.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into DFExplore, a 21 CFR Part 11-compliant data capture system provided by OCICB. The data system includes password

protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Collection and Storage of Biometric Data

In this study we are planning on using biometric data to be able to identify people who are study participants. 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

Particiants' 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.

11.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

11.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to FMPOS. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

11.9.1 Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

11.10 Publication and Data Sharing Policy

11.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

11.10.2 Genomic Data Sharing Plan

This study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

Appendix A: Toxicity Tables for Children

Local Reactogenicity Grading[1]

Local Reaction to	Mild	Moderate	Severe	Potentially Life

Injectable Product	(Grade 1)	(Grade 2)	(Grade 3)	Threatening (Grade 4)
Pain at site	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	ER visit or hospitalization
Erythema/Redness at site[2]	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Induration/Swelling at site[3]	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Bruising at site[2]	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Pruritus at Site	Does not interfere with activity	Repeated use of medication > 24 hours or interferes with activity	Prevents daily activity	ER visit or hospitalization
Limitation of Arm Movement	Does not interfere with activity	Repeated use of medication > 24 hours or interferes with activity	Prevents daily activity	ER visit or hospitalization

Abbreviations: ER, emergency room.

[1] The definitions provided in the table are modified versions taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007 and DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 2.1 July 2017.

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

[3] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Sign AE Grading[1]

Vital Signs[2]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever[3] (°C) **(°F)**	37.5 – 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; at rest + calm	121 – 135	136 – 150	>150	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[4]; at rest + calm	55 – 59	50 – 54	< 50	ER visit or hospitalization for arrhythmia
Hypertension - mm Hg; at rest + calm	NA	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) - mm Hg; at rest + calm	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	ER visit or hospitalization for hypotensive shock

Abbreviations: ER, emergency room.

[1] The definitions provided in the table are taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007 and the "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 1.0, December 2004, Clarification August 2009.

[2] Subject should be at rest for all vital sign measurements.

[3] Oral temperature; no recent hot or cold beverages or smoking.

[4] When resting heart rate is between 60 – 100 beats per minute

Systemic AE Grading[1]

Systemic AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
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 > 800 gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Nausea/ Vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Seizures- includes new or pre-existing febrile seizures	NA	NA	Seizure(s) that are not prolonged or repetitive	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)

Abbreviations: ER, emergency room; IV, intravenous; PO, "per os" or oral administration.

[1] The definitions provided in the table are modified versions taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007 and DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 2.1 July 2017.

Mali Laboratory AE Grading for Pediatric Population

Hematology and Biochemistry Values1, 2	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male/Female) - gm/dL	7.5 – 8.4	6.1 – 7.4	5.0 – 6.0	< 5.0 g/dL
WBC Increase - 10^3/µL	14.5 – 16.0	16.1 – 20.0	20.1 – 30.0	>30.0
WBC Decrease - 10^3/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0 with or without fever
Neutrophil/Granulocyte Decrease3 - 10^3/µL	0.75 – 0.99	0.50 – 0.74	< 0.50	< 0.50 with fever
Platelets Decreased - 103/µL	100 – 120	70 – 99	25 – 69	< 25
Creatinine (Male/Female) - µmol/L	95.00 – 119.99	120.00 – 149.99	150.00 – 200.00	> 200.00 and requires dialysis
Liver Function Tests/ALT - U/L	75.0 – 150.9	151.0 – 300.9	301.0 – 600.0	> 600.0

Abbreviations: ALT, alanine transaminase; WBC, white blood cell.

[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.

[3] Note, neutropenias are graded and followed, but based on previous experience in African populations, should be interpreted with caution since lower values are more frequently observed in people of African descent.(38, 39)

Appendix B: Institutional Normal Laboratory Values for Children

Hematology Reference Intervals*

	Reference Interval	Units
WBC- Leukocytes	4.8 – 11.2	$10^3/\mu L$
Hemoglobin	9.1 – 13.3	g/dL
Platelet Count	188– 551	$10^3/\mu L$
Absolute Granulocyte Count	1.4 – 5.9	$10^3/\mu L$

*Based on children ranging in age from 5 to 9 years old (based on sampling done at Bancoucmana and Doneguebougou research sites, Mali)

Biochemistry Reference Intervals*

	Reference Interval	Units
ALT	11.5 – 53.0	U/L
Creatinine	< 80.6	µM/L

*Based on children ranging in age from 5 to 9 years old (based on sampling done at Bancoucmana and Doneguebougou research sites, Mali)

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